NCTR Research Accomplishments and Plans

FY 1999-2000

Jefferson Laboratories of the FDA

LEADERS IN HEALTH SCIENCE RESEARCH FOR FDA
Jefferson, AR

The NCTR Office of Planning, Finance & Information Management compiles this document annually. To obtain additional information about the Center and/or additional copies of this document, you may contact the Division of Planning, 3900 NCTR Drive, HFT-321, Jefferson, AR 72079-9502; 870-543-7416 (phone); 870-543-7757 (fax); DBERANEK@NCTR.FDA.GOV (E-MAIL); http://www.fda.gov/nctr/ (World Wide Web).

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PREFACE

The National Center for Toxicological Research (NCTR), one of the six U.S. Food and Drug Administration's (FDA) product Centers and the Office of Regulatory Affairs (ORA), is dedicated to the conduct of fundamental and applied research to provide FDA with a stronger scientific base for making regulatory decisions. NCTR, a component of the Jefferson Laboratories of the FDA, is located in Jefferson, Arkansas, approximately thirty miles south of Little Rock.

The mission of the NCTR is to conduct peer-reviewed scientific research that supports the FDA's current and anticipated future regulatory needs. This involves fundamental and applied research specifically designed to define biological mechanisms of action underlying the toxicity of products regulated by the FDA and the development of improved methods for assessment of human exposure, susceptibility and risk. NCTR receives guidance and advice on the relevance and quality of its research programs from an extramural Science Advisory Board, its subcommittees, and liaison members from each of the other FDA centers and ORA.

FY1999 was highly productive for NCTR's eight research divisions, as evidenced by:

❶ The number of research articles published and the number of scientific staff invited to make presentations at national and international meetings.

❷ The significant contributions made toward the FDA Food Safety Initiative by development of: a) rapid methods for identifying food-borne pathogens; b) mechanistic studies on the acquisition of antibiotic resistance in microorganisms; and c) a monitoring program for the safety of microbial exclusion products in poultry.

❸ The development and validation of several new and improved transgenic animal and cellular models for *in vivo* and *in vitro* testing of carcinogens and mutagens.

❹ The development and staffing of a new phototoxicity laboratory. This unique facility will allow FDA and other government agencies to perform risk assessments on products whose toxicity may be related to exposure to light.

❺ The development of a simple innovative and inexpensive technology to measure freshness in seafood called FreshTag™.

NCTR has aggressively sought partnerships with other government agencies, academia, and industry. The success of this effort is indicated by:

❶ The fact that more than 30% of our publications have one or more co-authors from outside NCTR.

❷ Of particular importance was the increased support received from the National Institute for Environmental Health Sciences (NIEHS) to conduct bioassays, mechanistic studies, and risk assessments on a number of compounds of regulatory interest to both NIEHS and FDA.

❸ Also, with support from the FDA Office of Women's Health and the Chemical Manufacturers Association (CMA), NCTR developed a computerized Endocrine Disrupter Knowledge Base which will serve as a prototype for predicting activity of estrogens, androgens, neurotoxicants, and other toxic compounds.

Other important areas of research supported, in part, by outside funding included:

❶ Effects of anticonvulsants on complex brain functions in non-human primates.

❷ DNA chip array technology for determining polymorphisms of key metabolic genes.

❸ Development of a universal interface between HPLC instruments and mass spectrometers.

Perhaps of greater importance to our research accomplishments however, was the benefit gained by sharing knowledge through collaborations with scientific staff of other government, academic, and industrial institutions. I am proud to present this report that summarizes these and other NCTR research accomplishments and plans for the fiscal years 1999 - 2000.

Daniel A. Casciano, Ph.D.
Acting Director, NCTR

SCIENCE ADVISORY BOARD

SCIENCE ADVISORY BOARD
TO THE
NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

FUNCTION

One of the keys to maintaining a high quality research organization is the utilization of an outside body of experts, such as a Science Advisory Board (SAB), to periodically review the quality as well as the direction of the research. The NCTR SAB advises the Director in establishing, implementing and evaluating the research programs that assist the Commissioner of the Food and Drug Administration (FDA) in fulfilling regulatory responsibilities. This additional review ensures that the research programs at NCTR are scientifically sound and pertinent to the FDA.

FY1999 ACCOMPLISHMENTS

At its April 1999 meeting, the Board reviewed and approved the site visit reports of the Center's programs on Biochemical Toxicology, Genetic Toxicology and Molecular Epidemiology. It also received updates on the Board's recommendations for the Center's Biometry and Risk Assessment and Neurotoxicology programs.

In June 1999, a site visit of the Endocrine Disrupter Knowledge Base project was conducted. The report of this evaluation, along with the report of the site visit planned for the Center's Microbiology program conducted in November 1999 will be the subject of a spring 2000 meeting of the full Board.

The site visit reports and the minutes of the SAB meetings can be accessed at http://www.intranet.nctr.fda.gov/sab/.

In 1999, the Board lost by retirement, Drs. William (Bob) Bruce, University of Toronto, Tomas Guilarte, Johns Hopkins University, and Joseph Rodricks, ENVIRON International Corporation. Currently, recruitment is underway to fill these three vacancies as the Board begins its 26th year of service to the FDA and the Center.

SCIENCE ADVISORY BOARD TO THE NCTR
MEMBERSHIP ROSTER**

NAME/TITLE	AFFILIATION	TERM ENDS	EXPERTISE
Dr. Marion W. Anders* Professor, Chairman, Dept. of Pharmacology	University of Rochester Rochester, NY	6/30/00	Veterinary Medicine, Biochemistry/ Pharmacology
Dr. Robert E. Anderson Professor Emeritus, West Virginia University	WV School of Environmental Education, Inc. Bridgeport, WV	6/30/00	Food Technology
Dr. Catherine W. Donnelly Associate Dean, College of Agriculture & Life Sciences	University of Vermont Burlington, VT	6/30/02	Microbiology/Food Science
Dr. Stephen S. Hecht Wallin Land Grant Professor of Cancer Prevention	University of Minnesota Cancer Center Minneapolis, MN	06/30/02	Chemistry
Dr. Marcy E. Rosenkrantz Director Information Institute	Air Force Research Lab Rome, NY	6/30/00	Computational Chemistry
Dr. Charles L. Wilkins Distinguished Professor of Chemistry & Biochemistry	University of Arkansas 345 Campus Fayetteville, AR 72701	6/30/00	Chemistry
Mr. Ronald F. Coene Executive Secretary Deputy Director, Washington Operations, NCTR	FDA/NCTR Rockville, MD	Ongoing	Research Administration

* Serves as Chair of the Board
** Three new members are currently being processed for appointment to the Board

RESEARCH ACCOMPLISHMENTS AND PLANS

BIOMETRY AND RISK ASSESSMENT

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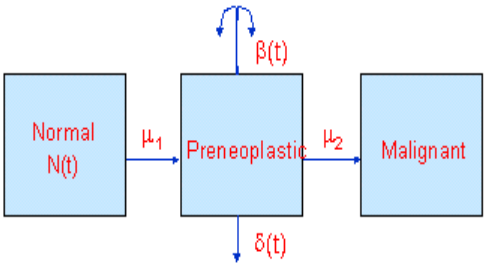
INTRODUCTION



$$P(t_0) = 1 - \exp\left\{ \int_0^{t_0} \mu_1(t) N(t) \, z(t) - 1] dt \right\}$$

- $z'(t) = -\beta(t)z(t)^2 + [\beta(t) + \delta(t) + \mu_2(t)]z(t) - \delta(t)$
- $z(t_0) = 1, t_0 > 0$ (e.g., t_0 = 104 weeks)

Cancer model used to predict the risk of liver tumors in $B_6C_3F_1$ mice exposed to Fumonisin B_1 in the diet.

The regulation of toxic substances in foods, drugs, biologics, cosmetics, animal drugs, and medical devices requires an engagement in risk assessment. Risk assessment is a process for determining the extent of human health hazard as a function of the conditions of exposure to toxic substances. It may involve the quantitative determination of estimates of risk corresponding to specific exposure levels, or it may simply involve a general determination that given exposure levels are sufficiently low to pose a negligible risk to those exposed. The daily dose rate, the route of exposure, the age at exposure, and the duration of exposure are all factors that influence risk.

The mission of the Division of Biometry and Risk Assessment is to conduct research to address FDA's regulatory need for new and improved methods of risk assessment; to assess the uncertainty associated with current approaches; and, to develop and apply new methods for the assessment of human exposure, susceptibility, and risk.

In most cases, assumptions must be made in order to extrapolate results observed at high doses in animal experiments to doses below the experimental range, to extrapolate across different routes and durations of exposure, and to translate animal risks (exposure levels) into human risks (exposure levels). Consequently, the uncertainty involved in estimating risks and in setting acceptable exposure levels can be substantial. Research is being conducted in the Division of Biometry and Risk Assessment to properly account for such uncertainty in the risk assessment process and, ultimately, to reduce this uncertainty. This research is directed toward the derivation of new methods as well as the assessment of current methods.

The research is relevant to FDA's strategic goals of improving the pre-market review process and of establishing strong post-market assurance standards. The research spans all of NCTR's strategic research goals: the development of knowledge bases; the development of new strategies for the prediction of toxicity; and the conduct of method-, agent-, and concept-driven research.

FY1999 ACCOMPLISHMENTS

Scientists in the Division of Biometry and Risk Assessment had 14 first-authored research papers accepted for publication, and co-authored an additional 12 papers. Major research accomplishments under each of NCTR's strategic research goals were as follows:

New Strategies for the Prediction of Toxicity

E06902.01. Research was conducted to evaluate the effect that shortening the two-year bioassay by as much as six months would have on the statistical power to detect carcinogenic human drugs. If the traditional bioassay can be shortened without loss of appreciable power, at least for some types of toxicants, then the Agency would benefit in terms of accelerating the drug approval process. During FY99 a manuscript providing guidance for when the bioassay might be shortened was submitted for publication.

E06908.01. Work continued on research to extend the two-stage, clonal-expansion model of carcinogenesis in order to develop biologically meaningful models to represent nonthreshold, threshold-like, and even U-shaped dose-response relationships for cancer. This research is intended to provide plausible dose-response models for the Agency to use when nonmonotone dose-response relationships for cancer are suggested by the weight of evidence. During FY99, two manuscripts were accepted for publication, a third was submitted, and two invited presentations were made.

E07037.01. A protocol was implemented to investigate the use of parametric density estimation for a mixture of normal densities to classify individuals with respect to variant hepatic CYP1A2 activity. Identifying genetic variants within the human population with respect to key enzymes in activation or detoxification pathways is important for evaluating relative disease risks for groups of people of varying susceptibility. In FY99, the protocol was revised and implemented, an invited presentation was made, and a manuscript on kernel density estimation for polymorphic populations was revised.

E07045.01. A protocol was finalized and implemented to conduct research on dose-response models for microbial risk assessment. Specific objectives are to develop improved models for estimating probabilities of microbial infection and disease, and to develop methods for formally incorporating model uncertainty into microbial risk assessment. This research is expected to give the Agency new quantitative tools to assess risk of disease following exposure to foodborne pathogens or contaminated medical devices, and to set acceptable exposure levels to microbes. In FY99, two invited presentations were made and two manuscripts were prepared for publication.

S00174. A project was undertaken to develop a state-of-the-art cancer model to describe and explain the tumor data observed in rats and mice in NCTR's feeding study on fumonisin B_1, a mycotoxin that occurs naturally in certain food products. The model will be provided to the Center for Safety and Applied Nutrition (CFSAN) and the Center for Veterinary Medicine (CVM) for potential use as a tool in their safety assessments of

FB_1. Mechanistic data on cell proliferation and apoptosis, dosimetry data on sphingolipid metabolism, and histopathology data on tumorigenicity are used simultaneously in the model to provide a biologically based predictive tool. During FY99, a comprehensive computer program was written in *Mathematica* to facilitate the fitting of various postulated models.

P00393. Research continued on the development of a multi-species pharmacokinetic database on dexamethasone, cocaine, and methylmercury to facilitate extrapolation from animals to humans. Interspecies extrapolation is a critical component of the safety assessment process for FDA-regulated products. Pharmacokinetics provides a way to improve dose-response extrapolation between species by utilizing parameters that reflect differences in dosimetry, thereby reducing uncertainty. In FY99, the database was populated with information from 234 publications on methylmercury pharmacokinetics, parameters were estimated using two different pharmacokinetic models, and a manuscript on interspecies comparisons was prepared.

Method-Driven Research

E06896.01. Research continued on the development of a statistical method for imputing (attributing) numbers of fatal and incidental tumors in each dose group in animal tumorigenicity studies. The method may be used to modify the Agency's preferred statistical test, the International Agency for Research on Cancer (IARC) test of Peto *et al*., to enable its use in the absence of pathologist-assigned cause of death or when cause of death is believed to be unreliable. During FY99, two manuscripts and one book chapter were accepted for publication.

E07009.01. Work continued on a protocol to develop statistical methods for testing multiple tumor sites, which control the experiment-wise false-positive error, while maintaining adequate power for detecting true carcinogenic effects. This research is aimed at alleviating pressure on the Agency from sponsors of regulated products to employ p-value adjustments that tend to downplay statistically significant effects at individual tumor sites. During FY99, two manuscripts were published, and two additional manuscripts were submitted for publication.

E07061.01. A protocol was submitted, revised and implemented to develop statistical methods for inferring effects on tumor frequencies and effects on time-to-observation for experiments in which multiple tumors of the same type are observed. This experiment will provide statistical methods for new experimental paradigms, such as photocarcinogenicity studies and skin-painting studies in transgenic mice, in order to improve the review of drug and cosmetic products that might either potentiate or inhibit the development of skin tumors. In FY99, one invited presentation was made and one manuscript was submitted for publication.

E07062.01. A protocol was developed, revised and implemented to develop statistical procedures for testing for bioequivalence of different formulations of the same drug or biologic based on dichotomous measures of effect. This research responds to FDA's

regulatory mission to ensure that different formulations of drugs and biologics are equally effective. During FY99, one manuscript was submitted for publication and an additional manuscript was begun.

P00393. Work continued on the development of mathematical models of human embryonic/fetal growth from implantation to birth. Good fits to growth data were obtained by nonlinear regression through numerical integration of an extended Gompertz model. One manuscript was accepted for publication during FY99.

S00032. Collaborative research was conducted with a number of external collaborators on statistical methods for mutagenicity assays involving transgenic rodents, extensions of the Armitage-Doll cancer model based on the ED_{01} data, risk estimation for developmental neurotoxicity, risk estimation for mixtures of chemicals, and calculating benchmark doses using change-point dose-response models. Several manuscripts are in preparation, and four were accepted for publication in FY99.

S00116. A chapter on risk assessment was finalized for a document being prepared by the National Research Council's Committee on Toxicology to provide guidance to NASA in developing spacecraft water exposure guidelines (SWEGs). The chapter espouses several new approaches to risk assessment, including the use of similar approaches for cancer and noncancer endpoints, the use of benchmark doses as points of departure, and a new way to combine multiple uncertainty factors.

Agent-Driven Research

E06957.01. Research was conducted on the quantification of differences in disposition of methadone in non-pregnant, pregnant, and post-partum rats. It is anticipated that the Agency's regulation of methadone and similar drugs will be enhanced by a fuller knowledge of differences in internal dosimetry that pose higher risks during pregnancy. During FY99, 30 data sets were simulated using an analog-digital hybrid computer to fit a two-compartment model and using the nonlinear mixed effect models (NONMEM) software. A manuscript comparing the results is in preparation.

E06957.11. Research was continued on the characterization of normal blood levels of clinical chemistry and hematology parameters in pregnant, untreated rats. Making these baseline data, which are noticeably absent from the open literature, available to the scientific community will enhance the interpretation of studies of regulated drugs. A manuscript characterizing 15 clinical chemistry parameters and eight hematology parameters was accepted for publication in FY99.

E07029.01. A protocol was implemented to evaluate mortality and disease incidence among atomic bomb survivors exposed to ionizing radiation *in utero* or as young children. This work, which is being done in collaboration with scientists at the Radiation Effects Research Foundation (RERF) in Japan, is expected to aid the Agency in regulating the use of certain medical devices on pregnant women. During FY99, three manuscripts were prepared and/or submitted for publication.

Concept-Driven Research

E00501-E00509. A major effort was continued to conduct a series of statistical analyses to evaluate the effects of caloric restriction on body weight, disease incidence and mortality in different species, genotypes and sexes, as part of NCTR's Project on Caloric Restriction. The results of these studies are expected to impact the way that caloric restriction and body-weight control are used to reduce variability in the long-term bioassay. During FY99, five manuscripts on general caloric-restriction issues and five manuscripts on the analysis of disease incidence and mortality have been begun, of which several have been submitted for publication.

FY1999 Interactions with FDA Centers

- Collaborated with scientists at CFSAN and CVM on the safety assessment of Fumonisin B_1.
- Fitted models to dose-response data on microorganisms for scientists at CFSAN and CDRH.
- Collaborated with scientists at the Center for Drug Evaluation and Research (CDER) on the development of statistical tests involving multiple tumor sites and statistical tests involving multiple tumors at the same site.
- Collaborated with scientists at CDER on evaluating the statistical implications of shortening the two-year bioassay in rodents.
- Collaborated with scientists at CDER on testing bioequivalence of different formulations of the same drug.
- Provided statistical consultation to scientists in the Office of Regulatory Affairs (ORA) on the comparison of methods for analyzing PCDDs/PCDFs.

FY1999 Center-wide Support

- Provided statistical consultation on a variety of experiments in support of the divisions of Genetic and Reproductive Toxicology and Molecular Epidemiology.
- Provided oversight to the statistical-analysis support group and the experimental-liaison support group under the Center-wide information-management contract, and reviewed all protocols for automated data processing requirements.
- Provided guidance to the Institutional Animal Care and Use Committee regarding statistical justifications of animal requirements for in-house experiments.
- Provided statistical guidance for the analysis of experiments conducted at NCTR under the FDA/NIEHS IAG as part of the National Toxicology Program.

Other FY1999 Accomplishments

Division scientists, through invited presentations at national and international meetings, workshops, universities, and other government agencies have broadened the impact of NCTR's research efforts to improve the risk assessment process. They have distinguished themselves as conference organizers, committee members, program reviewers, and associate editors of peer-reviewed scientific journals.

FY2000 GOALS

1. Develop statistical testing methods and predictive systems for identifying potential health hazards associated with toxic substances;

2. Develop biometrical methods for estimating risks associated with toxic substances to enable setting exposure levels that correctly reflect underlying uncertainties;

3. Develop mathematical models for better representation of internal exposure levels and biological mechanisms in order to reduce uncertainty in estimates of risk;

4. Provide statistical expertise to NCTR scientists on the design, conduct, and analysis of research studies to evaluate the toxicity of regulated products;

5. Assist other FDA centers in conducting risk assessments for the regulation of specific products and in investigating risk-assessment issues; and

6. Participate in interagency risk-assessment activities to maintain knowledge of the state-of-the-art and to promote the improvement and unification of risk-assessment practices across agencies.

FY2000 PLANS

All ongoing projects which have not been completed will continue into FY2000. In addition, some current projects will be expanded and several new projects will be initiated, as follows:

New Strategies for the Prediction of Toxicity

E06908.01. Work will continue to extend the two-stage clonal-expansion model of carcinogenesis. In particular, a manuscript on U-shaped dose-response curves for carcinogenesis will be finalized for publication. The regulatory implications of such U-shaped curves that can arise in theory even with genotoxic mechanisms or with background additivity that have long been considered indicators of low-dose linearity, will be discussed.

E07030.01. Research will resume on linking physiologically based pharmacokinetic (PBPK) models to biologically based dose-response (BBDR) models in order to improve

estimates of cancer risk. The full integration of models for target-tissue dose with models for target-tissue effect can be used to carry out complex mechanistic risk assessments like the one for Fumonisin B_1.

E07037.01. Methods will be developed to incorporate genetic considerations into mixture models for the distribution of CYP1A2 activities in human populations, in anticipation of providing a quantitative methodology for classifying individuals with respect to the risk of specific diseases based on enzyme profiles.

E07045.01. A manuscript on the development of microbial dose-response models and fitting these models to observed data on humans will be finalized and submitted for publication. A second manuscript on calculating statistical confidence limits for risk and dose, along with a procedure for incorporating model uncertainty into risk estimation will be finalized. Two invited presentations will be made at scientific meetings.

P00393. Research will continue on the use of a pharmacokinetic database for extrapolation from animals to humans. The database will be expanded to include dexamethasone pharmacokinetics, and modified rough-set theory will be used to examine the database.

S00174. Work will resume with colleagues at CFSAN on the joint project to investigate the feasibility of expanding the Threshold of Regulation for indirect food additives to include direct flavor additives. This approach has the potential to provide a defendable basis for a determination as to whether or not extensive toxicity testing is needed.

S00174. Work will continue on developing a state-of-the-art cancer model for the NCTR tumor data observed in rodents fed Fumonisin B_1. Preliminary results will be presented in an Agency-sponsored workshop on the Risk Assessment of Fumonisins. New data will be collected as needed, and subsequent modeling results will be presented to risk assessors at CFSAN and CVM.

X90074. A new protocol will be developed to motivate biologically relevant dose-response models of bacterial survival in the stomach, growth in the intestine, and infection in the blood, by conducting well-defined experiments in rodents. It is anticipated that the Agency's participation in the Food Safety Initiative will be enhanced by the experimental motivation of mathematical dose-response models for microbial risk assessment, by reducing uncertainties associated with internal dose, host susceptibility and microorganism infectivity. This protocol will be developed in collaboration with colleagues at CFSAN and at the U.S. EPA.

Method-Driven Research

E06896.01. Work will continue on improving the statistical method for imputing the numbers of fatal and incidental tumors needed to modify the cause-of-death test for evaluating tumorigenicity data. It is anticipated that a manuscript describing the method will be accepted for publication in FY2000. If so, this would provide an alternative way to compute CDER's preferred test that would eliminate controversy surrounding the issue of pathologist-assigned cause of death.

E07009.01. Research will continue on statistical tests involving multiple endpoints. Manuscripts on a weighted adjustment method for controlling the family-wise error rate and global test statistics for subsets of multiple endpoints will be finalized for publication. In addition, a new method to assign weights to p-values based on the correlation structure of the endpoints will be developed.

E07061.01. Research will continue on statistical tests for increased frequencies of tumors and/or decreased latency between treatment groups. Computational procedures to analyze phototoxicity data will be developed, and a manuscript on testing for dose-related trend will be prepared.

E07062.01. A manuscript on testing the equivalence of two proportions will be finalized, and manuscripts on testing equivalence using the logistic regression trend test and testing equivalence of two multinomial proportions will be developed.

S00032. Collaborative research with external investigators will continue on estimating risks and calculating benchmark doses for nonquantal toxicity data, including developmental neurotoxicity data, data involving mixtures of populations and change-point dose-response data. In addition, collaborative efforts to develop statistical methods for mutagenicity assays involving transgenic rodents and to extend the Armitage-Doll cancer model will continue.

Agent-Driven Research

E06957.01. Work will continue on modeling changes in the disposition of methadone in pregnant rats and their fetuses. A manuscript comparing simulation using a hybrid analog-digital computer to simulation using the nonlinear mixed effect models (NONMEM) software package will be finalized.

E07029.01. Collaborative research with scientists at the Radiation Effects Research Foundation in Japan will continue to assess mortality follow-up data for the effects of *in utero* exposure from atomic bombings in Japan. Three manuscripts will be finalized on risk assessment of childhood cancer, lifetime cancer incidence, and persistent inflammation.

Concept-Driven Research

E00501-E00509. Statistical analyses will be completed on the series of experiments conducted under the Project on Caloric Restriction. The remaining within-study and between-study comparisons will be made and three additional manuscripts comparing the effects of caloric restriction between sexes, genotypes, and species will be submitted for publication.

PUBLIC HEALTH SIGNIFICANCE

The Division of Biometry and Risk Assessment is a focal point within the FDA for research in the area of Health Risk Assessment. Human health risk estimates impact the regulation of exposure to toxic substances, thereby affecting both the health of the U.S. population and the U.S. economy. The Division of Biometry and Risk Assessment has the mission of identifying uncertainties in the risk assessment process, and developing risk estimation techniques that either appropriately account for or reduce these uncertainties. The ultimate goal of the research is to improve the regulation of natural or synthetic toxic substances occurring in foods, drugs, biologics, cosmetics, animal drugs, and medical devices. Continued significance to the FDA is fostered through interactions with individuals and committees at other FDA centers that are involved in evaluations of risk for the regulation of specific products.

ACTIVE PROJECTS FY1999

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0049400	**Caloric Restriction in Fischer 344 Rats** 1. Evaluate the logistics and performance of the newly developed rat and mouse cages; 2. Evaluate the use of known weight pellets in producing a calorically restricted diet; 3. Derive practical procedures with the new equipment; 4. Estimate the 100% consumption values for mice and fats fed ad libitum, including factors such as wastage; 5. Optimize study conditions, such as procedures, cages, etc., for maximum efficiency.	Turturro, Angelo*
E0050100 thru E0050700	**NIA-IAG Caloric Restriction and Aging** Breed and age calorically restricted rodents.	Turturro, Angelo*
E0050900	**F-344 Rat Fed Ralston-Purina Masoro Mod. Diet** Selected diet for caloric restriction.	Turturro, Angelo* Gaylor, David W. Hart, Ronald W. Sheldon, Winslow G.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0689601	**Attribution of Tumor Lethality in the Absence of Cause-of-Death Information** To develop a nonparametric procedure for estimating distributions of time to onset of and time to death from occult tumors in the absence of cause-of-death information. To develop a method for entering the number of fatal tumors in an experiment that lacks cause-of-death data, in order to modify the International Agency for Research on Cancer (IARC) cause-of-death test. To develop a procedure for estimating the lag time between onset of and death from an occult tumor, when cause-of-death data are unavailable To illustrate the new procedures using data from the Project on Caloric Restriction (PCR) studies.	Kodell, Ralph L.* Ahn, Hongshik
E0690201	**Bioassays of Shortened Duration for Drugs: Statistical Implications** To conduct a Monte Carlo simulation study to evaluate the effect that terminating rodent bioassays at 18 months (or earlier) instead of 24 months would have on the statistical power to detect carcinogenic human drugs.	Kodell, Ralph L.* Chen, James J. Lin, Karl K.
E0690801	**Properties of the Hazard and Survival Functions of the MVK Stochastic Carcinogenesis Model** Investigate mathematical properties of the Moolgavkar-Knudson-Venzon (MVK) stochastic carcinogenesis model to deepen understanding and enlarge applicability of the MVK model. Study the two most important quantities of this model: the hazard and the survival function. Study the joint distributional properties of the numbers of initiated and malignant cells; develop parameter estimation procedures so that the model can be fitted to real data; exploit possible generalizations and extensions of this model.	Zheng, Qi* Kodell, Ralph L.
E0695301	**Rodent Embryo and Fetal Sectioning for Three-Dimensional Image Reconstruction and Animation** To develop the staining and sectioning techniques for conventional and laser scanning confocal microscopy to produce electronic images of rodent embryos and fetuses that can be used for computerized image morphing, 3D reconstruction, and animation.	Young, John F.* Bolon, Brad N. Branham, William S. Haas, Andy Meehan, Joseph F. Sheehan, Daniel M. Warbritton, Alan R.
E0695401	**Resting Metabolic Rate, Body Composition, and Dietary Assessment** 1. Develop a prediction model for the resting metabolic rate (RMR) based on anthropometric data and body composition, and validated by measured RMR; 2. Collect dietary intake data and maximize their accuracy; identify potential sources of reporting of bias in relation to anthropometric data, and examine the correlation of calorie intake with RMR.	Freni, Stan C.*
E0698401	**Statistical Analysis and Characterization of the Joint Actions of Toxicants** To develop a procedure for analyzing the quantal response data from a mixture experiment at a fixed total concentration; to develop a procedure for analyzing the survival data from a mixture experiment at a fixed total concentration; To develop a mixture model including both proportions and total concentrations; To apply the proportion-concentration model to characterize the joint actions of toxicants.	Chen, James J.* Kodell, Ralph L. Zheng, Qi

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0700901	**Analysis of Multiple Tumor Sites** 1. To develop analytical and numerical techniques for computing the experiment-wise error rate in testing of multiple tumor sites; 2. To evaluate and compare the experiment wise error rate and power of various methods of p-value adjustment and recommend an optimal method for test of site-specific effects; 3. To evaluate the experiment wise error rate and power of global statistics for an overall test of carcinogenicity; 4. To recommend optimal procedures, which control the experiment wise error rate and still maintain the power, for the analysis of multiple tumor sites.	Arani, Ramin B.* Chen, James J. Macgregor, Jim
E0702901	**Mortality Among Atomic Bomb Survivors who were Exposed *In Utero*** 1. To estimate the dose-response relationship between non-cancer mortality and radiation exposure; 2. To assess the effect of gestational age at exposure on mortality; 3. To appraise the role of severe mental retardation in mortality.	Delongchamp, Robert R.* Feigal, David
E0703001	**Combining Carcinogenesis Models with Pharmacokinetic Models** 1. Explore methods for using physiologically based pharmacokinetic models as tools for allowing target dose to be directly incorporated into stochastic carcinogenesis models, and hence improve risk assessment for various kinds of carcinogenic chemicals; 2. Within the context of using combined models, investigate the feasibility of estimating certain biological parameters from data, if such parameter values are not readily available in the literature.	Zheng, Qi* Kodell, Ralph L.
E0703701	**A Mixture Model Approach to Classifying CYP1A2 Variants that Adjusts for their Current Smoking Status** To examine statistical methods for parametric density estimation based upon a mixture of normal distributions; To apply the method to a data set where hepatic cytochrome P4501A2 activity appears to be induced by smoking cigarettes.	Delongchamp, Robert R.* Chen, James J. Lang, Nicholas P. Lung-An, L
E0704501	**Dose-Response Modeling for Microbial Risk Assessment** 1. To evaluate existing dose-response models for microbial risk assessment; 2. To develop improved models for estimating probabilities of infection and disease; 3. To develop methods for incorporating model uncertainty into microbial risk assessment.	Kang, Seung-ho* Chen, James J. Kodell, Ralph L.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0706101	**Statistical Analysis of Tumor Multiplicity Data** 1. To investigate the model of Kokoska, et al. For analyzing tumor multiplicity data from single-induction experiments, using the negative binomial distribution for the number of induced tumors and the Weibull distribution for the time to observation of such tumors; 2. To develop a likelihood-ratio approach, adapted from the model of Kokoska, et. al. for testing between-group differences with respect to the expected number of induced tumors as well as the distribution of time to observation; 3. To develop tests for dose-related trend with respect to the expected number of induced tumors and the distribution of time to observation; 4. To extend the model to situations involving multiple or continous dosing, and situations in which there is a background of spontaneous tumors; 5. To conduct a Monte Carlo simulation study to compare the new methodology to conventional analytical approaches, and to evaluate its robustness and identifiability; 6. To develop user-friendly software for easy implementation of the proposed analytical procedures.	Kodell, Ralph L.* Chen, James J. Delongchamp, Robert R. Lin, Karl K.

PROJECTS COMPLETED FY1999

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0697401	**Collinearity Under Proportional Hazards Model** (i) To provide diagnostic tools to detect the presence of collinearity under proportional hazards and its quantitative effect on the results. (ii) To provide algorithms to combat the harmful influence of collinearity, i.e., stabilize the parameter estimates and their variance. (iii) To conduct a simulation study to examine the effectiveness of the algorithms.	Arani, Ramin B. * Chen, James J. Bove, Celeste

FY1999 PUBLICATIONS*

1. Aidoo, A., Desai, V.G., Lyn-Cook, L.E., Chen, J.J., Feuers, R.J. and Casciano, D.A. Attenuation of bleomycin-induced Hprt mutant frequency in female and male rats by calorie restriction. Mutation Research, Accepted: 9/24/99. **(Collaborating with Gen. & Repro. Tox.) (E0699101)**

2. Ahn, H., Chen, J.J. and Chang, J.Y. On sequential testing procedures for a dose-response analysis. 1999 Proceedings of the American Statistical Association Joint Meetings, Accepted: 8/9/99. **(E0700901)**

3. Ahn, H. and Kodell, R.L. Analysis of animal carcinogenicity data using S-PLUS. In: S-PLUS in the Pharmaceutical Industry, Accepted: 3/24/99. **(E0689601)**

4. Ahn, H., Kodell, R.L. and Moon, H. Attribution of tumor lethality and estimation of time to onset of occult tumors in the absence of cause-of-death information. Applied Statistics, Accepted: 6/25/99. **(E0689601)**

** (_ _ _ _ _ _ _) Indicates the related NCTR project number; NA-Not Applicable*

5. Chen, J.J. and Kodell, R.L. A decision-tree strategy for combining diagnostic tests for prediction. Biometrical Journal, Accepted: 1/18/99. **(S00032)**

6. Delongchamp, R.R., Chen, J.B., Heflich, R.H. and Malling, H. An estimator of the mutant frequency in assays using transgenic animals. Mutation Research, 440:101-108, 1999, Accepted: 1/6/99. **(E0697701)**

7. Gaylor, D.W. and Kodell, R.L. Dose-response trend tests for tumorigenesis, adjusted for body weight. Toxicological Sciences, 49:318-323, 1999, Accepted: 10/29/98. 1999 **(Collaborating with Ofc. of Dir./Imm. Ofc.) (E0691101)**

8. Gaylor, D.W. and Kodell, R.L. Percentiles of the product of uncertainty factors for establishing probabilistic reference doses. Risk Analysis, Accepted: 8/2/99. **(Collaborating with Ofc. of Dir./Imm. Ofc.) (S00116)**

9. Gross, M., Anderson, K., Lang, N.P. and Delongchamp, R.R. The distribution and concordance of N-acetyltransferase genotupe and phenotype in an American population. Cancer Epidemiology, Biomarkers & Prevention, Accepted: 2/1/99. **(Collaborating with Mol. Epi.) (E0694601)**

10. Hansen, D.K., Young, J.F., Laborde, J.B., Wall, K.S. and Holson, B. Pharmacokinetic considerations of dexamethasone-induced developmental toxicity in rats. J. Toxicological Sciences, 48:230-239, 1999, Accepted: 5/21/99. **(Collaborating with Gen. & Repro. Tox.) (E0663812)**

11. Hart, R.W., Bucci, T.J., Turturro, A., Leakey, J.E., Feuers, R.J., Duffy, P.H., James, S.J., Lyn-Cook, B.A., Pipkin, J.L. and Li, S. Caloric intake as a modulator of carcinogenicity and anticarcinogenicity. In: Carcinogenic/ Anticarcinogenic Factors in Food: Novel Concepts, Accepted: 3/12/99. **(Collaborating with Ofc. of Dep. Dir.) (E0260112)**

12. Hart, R.W., Duffy, P.H., Fu, P.P., Leakey, J.E., Seng, J.E., Turturro, A. and Li, S. The interaction of energy metabolism with xenobiotic pathways. In: Energy Metabolism and Carcinogenesis, Accepted: 5/1/99. **(Collaborating with Ofc. of Dir./Imm. Ofc.) (E0699801)**

13. Hart, R.W., Seng, J.E., Turturro, A., Leakey, J.E., Feuers, R.J., Duffy, P.H., Buffington, C.K., Cowan, G.S., Lewis, S.M., Pipkin, J.L. and Li, Erdong. Adaptive role of caloric intake on the degenerative disease processes. Toxicological Sciences, Accepted: 9/1/99. **(Collaborating with Ofc. of Dir./Imm. Ofc.) (E0692401)**

14. Hwang, J. and Chen, J.J. An evaluation of risk estimation procedures for mixtures of carcinogens, Risk Analysis, Accepted: 3/10/99. **(E0698401)**

15. Kang, S. An optimal property of exact multinomial test and Fisher's Exact Test. Statistics and Probability - Letters, Accepted: 12/8/98. **(NA)**

16. Kang, S. Exact likelihood ratio test of independence of binary responses within clusters. Computational Statistics & Data Analysis, Accepted: 5/25/99. **(NA)**

17. Kodell, R.L. and Gaylor, D.W. Combining uncertainty factors in deriving human exposure levels of noncarcinogenic toxicants. Annals of the New York Academy of Sciences, Accepted: 10/1/98. **(NA)**

18. Kodell, R.L., Ahn, H. and Moon, H. Comparing age-specific tumor incidence rates when cause of death is not assigned. The XIXth International Biometrics Conference: Invited Papers, Accepted: 11/18/98. **(E0689601)**

19. Laborde, J.B., Wall, K.S., Bolon, B.N., Kumpe, T.S., Patton, R.E., Zheng, Q., Kodell, R.L. and Young, J.F. Haematology and serum chemistry parameters of the pregnant rat. Laboratory Animals, 33:275-287, 1999. Accepted: 2/8/99. **(Collaborating with Gen. & Repro. Tox.) (E0695711)**

20. Luecke, R., Wosilait, W.D. and Young, J.F. Mathematical modeling of human embryonic and fetal growth rates. Growth, Development & Aging, Accepted: 7/4/99. **(S00116)**

21. Moon, H., Ahn, H., Kodell, R.L. and Pearce, B.A. A comparison of a mixture likelihood method and the EM algorithm for an estimation problem in animal carcinogenicity studies. Computational Statistics and Data Analysis, 31:227-238, 1999. Accepted: 1/22/99. **(E0689601)**

22. Pipkin, J.L., Hinson, W.G., Young, J.F., Rowland, K.L., Shaddock, J.G., Tolleson, W.H. and Casciano, D.A. Induction of stress proteins by electromagnetic fields in cultured HL-60 cells, Bioelectromagnetics, 20:347-357, 1999. Accepted: 10/28/98. **(Collaborating with Gen. & Repro. Tox.) (E0677000)**

23. Razzaghi, M., Kodell, R.L. and Allen, M.E. Risk assessment for quantitative responses using a mixture model. Biometrics, Accepted: 6/15/99. **(Collaborating with R.O.W. Sciences) (S00116)**

24. Turturro, A., Duffy, P.H. and Hart, R.W. Antioxidation and evolution: Dietary restriction and alterations in molecular processes. In: *Antioxidants in Human Health and Disease*, Accepted: 2/18/99. **(E0050400)**

25. Turturro, A., Hass, B.S. and Hart, R.W.. Hormesis - Implications for risk assessment caloric intake (body weight) as an exemplar. Human and Experimental Toxicology, 17(8):454-459, 1999. Accepted: 10/1/98. **(NA)**

26. Turturro, A., Witt, W.M., Lewis, S.M., Hass, B.S., Lipman, R. and Hart, R.W. Growth curves and survival characteristics of the animals used in the biomarkers of aging program. Journal of Gerontology, Accepted: 6/28/99. **(E0050400)**

27. West, R. and Kodell, R.L. A comparison of methods of benchmark dose estimation for continuous response data. Risk Analysis, 19(3):453-459, 1999. Accepted: 10/17/98. **(S00116)**

28. Wolff, G.L., Kodell, R.L., Kaput, J.A. and Visek, W.J. Caloric restriction abolishes enhanced metabolic efficiency induced by ectopic agouti protein in yellow mice. Proceedings of the Society for Experimental Biology and Medicine, 221:99, 1999. Accepted: 1/13/99. **(Collaborating with Biochem. Tox.) (E0260301)**

29. Zheng, Q. Automating the computation of cumulants of stochastic population processes. Proceedings of the 31st Symposium on the Interface, Accepted: 7/1/99. **(NA)**

30. Zheng, Q. Comments on the hazard fundtion of a two-stage carcinogenesis model. Radiation Research, 151:120, 1999. Accepted: 11/12/98. **(E0690801)**

31. Zheng, Q. Computing the probability and the hazard of developing a detectable tumor. Proceedings of the 1999 JSM of the American Statistical Association, Accepted: 8/17/99. **(E0690801)**

32. Zheng, Q. Progress of a half century in the study of the Luria-Delbruck distribution, Mathematical Biosciences, Accepted: 8/19/99. **(E0690801)**

33. Zheng, Q. Solution to the hazard function of some two-stage carcinogenesis models when normal cell growth is piecewise linear. Communications in Statistics - Theory and Methods, 29(8):1921-1929, 1999. Accepted: 4/19/99. **(S00116)**

BIOCHEMICAL TOXICOLOGY

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INTRODUCTION

The liquid handling system shown is configured to perform automated on-line sample preparation coupled with HPLC and electrospray mass spectrometry for high throughput quantitative analysis of carcinogen-DNA adducts.

The Division of Biochemical Toxicology conducts fundamental and applied research specifically designed to define the biological mechanisms of action underlying the toxicity of products either regulated by or of interest to the Food and Drug Administration (FDA). This research centers on assessing the toxicities and carcinogenic risk associated with specific chemicals and gene-nutrient interactions, and the introduction of new techniques to assess toxicities and carcinogenic risk. The risk assessment research is firmly rooted in mechanistic studies focused on the understanding of toxicological endpoints, an approach that allows greater confidence in the subsequent carcinogenic risk assessments. Research within the Division capitalizes on scientific knowledge in the areas of biochemistry, organic chemistry, cellular and molecular biology, immunology, nutritional biochemistry, and pharmacology. It is supported by sound technical skills, the availability of state-of-the-art equipment, and internal and external collaborations and funding.

FY1999 ACCOMPLISHMENTS

In 1999, the Division conducted research on compounds nominated by the FDA for evaluation by the National Institute of Environmental Health Sciences, National Toxicology Program (NIEHS/NTP). This emphasis reflects the fact that the NCTR has superb animal facilities supported by a staff of scientists with strong multidisciplined mechanistic research experience; as such, the Center has the capability to conduct subchronic and chronic toxicological assessments in a rigorous manner to address the FDA's needs. While acknowledging the limitations of animal bioassays, these studies currently serve as the benchmark by which toxicological assessments are made by federal agencies, including the FDA. In addition to providing basic information on toxicological endpoints, such as cancer, these experiments form the basis for mechanistic studies to ascertain if the response detected in the experimental model is pertinent to humans.

The Division's NIEHS/NTP investigations have focused on the mycotoxin fumonisin B_1, which was nominated by the Center for Food Safety and Applied Nutrition (CFSAN); the pediatric sedative chloral hydrate, in response to a request made by the Center for Drug Evaluation and Research (CDER); malachite green, a therapeutic agent used in aquaculture that was nominated by the Center for Veterinary Medicine (CVM); and ethanol and urethane, at the request of CFSAN to assist this center to establish regulatory levels for the carcinogen urethane in food products.

During the year, the final report on the fumonisin B_1 bioassay was completed and approved by the NTP Technical Reports Review Committee. The study determined that fumonisin B_1 is a kidney carcinogen in male rats and a liver carcinogen in female mice. The mechanism of action of fumonisin B_1 was also intensely investigated and the data indicate that this mycotoxin interrupts *de novo* sphingolipid synthesis through inhibition of ceramide synthase. This enzymatic inhibition results in apoptotic cell death, which suggests that tumor formation arises as a result of compensatory regeneration. These results are being used by staff members in the Division of Biometry and Risk Assessment in conjuction with scientists from the Division of Biochemical Toxicology and CFSAN to develop a risk assessment for dietary exposures to fumonisin B_1.

The bioassay on the pediatric sedative chloral hydrate was completed during the year and the final report is being prepared for presentation to the NTP in May 2000. The bioassay results will be combined with metabolism and cell proliferation data, as well as results from other mechanistic studies, to develop a comprehensive risk assessment for this drug.

Bioassays for malachite green were initiated during 1999. These studies have focused on both malachite green and its reduction product leucomalachite, the major metabolite found in edible tissues of fish exposed to malachite green. In addition to the bioassays, mechanistic studies were conducted with emphasis on understanding the metabolic pathways that lead to the DNA adducts that have been detected *in vivo* from malachite green and leucomalachite green.

The chronic bioassay phase of the urethane and ethanol bioassay was completed during the year and the tissues are currently undergoing pathological examination. Mechanistic studies associated with this project have focused on developing methodologies based upon both ^{32}P-postlabeling and mass spectrometry to detect the etheno-DNA adducts that are suspected to arise from urethane. Studies were also started to examine the DNA adducts formed from the pyrrolizidine alkaloid riddelliine, a compound of interest to CFSAN that is undergoing a NTP-sponsored chronic bioassay. To date, one adduct present in the livers of riddelliine-treated rats has been identified.

As part of the NIEHS/NTP effort, a series of studies on endocrine-active compounds was started to assess the effects of environmental endocrine-disrupting chemicals on reproduction and carcinogenesis over multiple generations. The studies utilize a variety of functional and structural endpoints to assess reproductive and developmental outcomes and to define more accurately the dose-response relationships for these

chemicals. The compounds being examined are genistein, ethinyl estradiol, nonylphenol, and methoxychlor, which were selected because of their estrogenic activities, and vinclozolin, a fungicide with anti-androgenic activity. An important aspect of the studies is that they are being conducted using a diet that has very low levels of major phytoestrogens (genistein, daidzein, and coumestrol). This should have broad implications for reproductive toxicity studies in animals and studies of endocrine-active compounds. During 1999, all of the dose-range-finding studies were completed. In addition, multigeneration studies with genistein and nonylphenol were begun and pilot studies for the genistein-mammary tumor study were completed.

Phototoxicity is another area of interest to the Agency that was recently funded by the NIEHS/NTP. This investigation was initiated in response to CFSAN's concern about the potential interaction between UV light and over-the-counter cosmetics containing α-hydroxy acids. A phototoxicology research facility with the capability of performing bioassays did not exist within the FDA. During the year, such a facility was constructed that will allow mice to be exposed to either simulated solar light or fluorescent tube-generated light. The experimental model selected for these studies is the SKH-1 hairless mouse and during 1999, the effects of skin creams containing α- and β-hydroxy acids upon the skin of these mice was investigated.

Traditional chronic carcinogenicity bioassays are both very expensive and lengthy; thus, the development of alternative methods of assessing carcinogenic potential should be of great value. One approach that is currently being investigated is the neonatal mouse tumorigenicity assay. The advantages of this method are that only limited amounts of test material are required, a direct assessment is obtained as to whether or not the agent acts through a genotoxic mechanism, and less time is required to elicit a carcinogenic response. In collaboration with investigators at CDER, this alternative bioassay has been applied to benzodiazepines, antihistamines, lipid peroxidation products, estrogens, antiestrogens, peroxisome proliferators, and lipid peroxidation inducers. Similar studies have been conducted with antiretroviral nucleoside analogues, such as azidothymidine.

An ongoing goal within the Division is to exploit both the immunogenicity and the antigenicity of toxicants, metabolites, and DNA adducts to develop and apply immunochemical methods combined with mass spectral techniques to address problems of regulatory concern. This technology has been applied to fumonisin B_1, fumonisin B_2, and fumonisin B_3, aromatic amine DNA adducts, nucleoside analogues of anti-HIV drugs, and etheno-type DNA adducts formed by urethane. More recently, studies have been initiated to prepare antibodies against UV photoproducts in support of the Division's phototoxicity effort. In addition, Division investigators have developed methodologies to assay hydroxylation of endogenous estrogens. This work, which is funded by the FDA's Office of Women's Health, is an outgrowth of recent clinical and experimental data that have linked differences in 2- and 4-hydroxylation of endogenous estrogens with differences in the risk of developing breast cancer.

A major focus of the Division is to develop analytical methodology based on mass spectrometry to measure biomarkers of exposure and toxicity in animals and humans in conjunction with studies that define mechanisms of toxicity. Toward this end, liquid chromatography-mass spectrometry (LC/MS) methods were developed and applied to analyze the soy isoflavones genistein and daidzein in serum and tissues from neonatal, prepubertal, and adult rats as part of the Division's ongoing endocrine disrupter studies. Likewise, mechanistic studies with genistein, daidzein, and equol indicated that these compounds are suicide substrates for the inactivation of thyroid peroxidase, the enzyme that catalyzes synthesis of thyroid hormones. Additional LC/MS methods were developed for the sensitive and selective detection of DNA adducts formed through metabolic activation of exogenous chemical carcinogens and through byproducts of normal aerobic metabolism.

A strong emphasis within the Division has been in the area of nutritional folic acid deficiency. As part of this program, Division investigators have evaluated the progression of global DNA hypomethylation and promoter region hypermethylation in the p53 gene. The results offer an alternative mechanism for p53 inactivation in cancers that do not have p53 mutations. These investigators have also developed a new HPLC method to measure thiol metabolites associated with folate-dependent homocysteine metabolism. Using this methodology, they have shown that increased plasma homocysteine, a risk factor for cardiovascular disease and certain birth defects, is associated with a parallel increase in S-adenosylhomocysteine. In studies funded by the FDA's Office of Women's Health, this group found abnormal folate metabolism to be associated with polymorphisms in the methylene tetrahydrofolate reductase and methionine synthase reductase genes in mothers of children with Down syndrome.

FY2000 PLANS

During 2000, the final report on the NTP-nominated chemical chloral hydrate will be completed. The results from the chronic bioassay on the interactions of urethane and ethanol will be compiled, and the chronic bioassay with malachite green will continue. The multigeneration reproductive assessments of genistein and nonylphenol will be completed, and studies with ethinyl estradiol and methoxychlor will be started. In the area of phototoxicity, efforts will focus upon quantifying the effects of creams containing α- and β-hydroxy acids on the induction of edema, apoptotic cell death, and basal cell proliferation in mice exposed to UV and simulated solar light. These experiments will serve as the foundation for the design of photocarcinogenesis tumor studies.

Mechanistic studies will continue on fumonisin B_1, malachite green, urethane in the presence of alcohol, and endocrine-disrupting chemicals. The fumonisin B_1 experiments will be centered on the isolation and characterization of ceramide synthase, a key enzyme involved in the toxicities of fumonisin B_1. In addition, results will be compiled from a short-term study with a number of fumonisin derivatives (fumonisin B_1, fumonisin B_2, fumonisin B_3, hydrolyzed fumonisin B_1, 2-hydroxypyridinyl fumonisin B_1, and carboxymethyl fumonisin B_1) to ascertain their contributions to the

toxicities associated with *Fusarium*. Studies with malachite green will focus on the importance of DNA adduct formation in the suspected tumorigenicity of the dye and on the metabolic pathways leading to these adducts. Experiments with urethane and ethanol will emphasize the DNA adducts formed by urethane and how these are affected by increasing concentrations of ethanol. Mechanistic studies with endocrine-disrupting chemicals will include investigating the effects of dietary genistein on the growth of chemically-induced mammary tumors, and determining the effects of various endocrine-disrupting chemicals on steroid metabolizing enzymes and estrogen receptors. Experiments with riddelliine will focus on determining dose-response relationships and determining if the DNA adduct formed from this compound is also present in animals treated with other carcinogenic pyrrolizidine alkaloids.

Experiments will continue on tamoxifen, an important adjunct chemotherapeutic agent for treating women with breast cancer. These studies will characterize DNA adducts from tamoxifen metabolites and analogues, and develop methods for their detection and quantitation. Experiments will also be conducted to determine if tamoxifen or its derivatives increases the frequency of mutations at the *Hprt* gene and if these mutations can be used as an indicator for the potential genotoxicity of the antiestrogen. Studies are also planned to use the recently developed methodologies to determine if the same types of DNA adducts formed in rodents are found in women undergoing treatment with tamoxifen.

Despite the acknowledged importance to human cancer prevention, the specific nutrient-gene interactions that promote malignant transformation are not yet understood. Chronic deficiencies in the methyl donors folate, methionine, and choline reproducibly induce liver tumors, reduce total folate levels, and alter distribution of folate derivatives. Since adequate folate availability is essential for maintenance of *both* deoxynucleotide DNA precursor pools and normal DNA methylation, experiments will continue to study the effect of folate deficiency on deoxynucleotide pool balance, base misincorporation, cell death, and the progressive alterations in p53 gene methylation patterns and expression during liver tumor progression with chronic folate/methyl deficiency.

As noted earlier, abnormal folate metabolism is associated with polymorphisms in the methylene tetrahydrofolate reductase and methionine synthase reductase gene in mothers of children with Down syndrome. This suggests that DNA hypomethylation secondary to inadequate maternal folate status may contribute to abnormal chromosome segregation and nondisjunction of chromosome 21. Studies are planned to test this hypothesis. In addition, studies of gene-nutrient interactions in the risk of carcinogenesis and birth defects are planned.

During the year, final reports will be completed of newborn mouse bioassays on specific classes of chemicals including estrogens, antiestrogens, peroxisome proliferators, and lipid peroxidation inducers. Bioassays results will be compiled on antiretroviral nucleoside analogues. In addition, the types of mutations induced by the antiretroviral nucleoside analogues will be determined. Bioassays will be initiated on a series of

known human carcinogens as well as other compounds of interest to the Agency. These studies will provide critical information on the strengths and limitations of the newborn mouse bioassay.

Lipid peroxidation generates a number of products, such as malondialdehyde, formaldehyde, acetaldehyde, acrolein, and crotonaldehyde. Several of these lipid peroxidation products have been shown to be highly cytotoxic, genotoxic, mutagenic, and tumorigenic in rodents. In addition, they have been found to have the capability to bind covalently to cellular DNA, forming the endogenous DNA adducts that have been detected in experimental animals and human tissues. Experiments will continue to develop sensitive and reliable analytical methodology for detecting and quantifying these endogenous DNA adducts in animal and human samples.

Estrogen exposure is a major risk factor for breast cancer; however, the relative importance of competing pathways of estrogen metabolism to the risk of breast cancer has not been determined. The metabolism of estrone and estradiol to catechols can follow one of two pathways, 2-hydroxylation to form 2-hydroxy estrogens or 4-hydroxylation to give 4-hydroxy estrogens. The critical importance of these alternative catechol pathways is reflected in the extensive literature associating the tendency towards 4-hydroxylation with estrogen-induced carcinogenesis and shift towards 2-hydroxylation with reduced risk or even protection. Studies in this area have been funded by the FDA's Office of Women's Health and the Arkansas Breast Cancer Research Program and will focus on improving HPLC procedures for separating estrogen metabolites and synthesizing immunogens that will be used to develop immunochemical methods for metabolite enrichment.

PUBLIC HEALTH SIGNIFICANCE

The FDA is entrusted with the responsibility of ensuring the safety of foods, drugs, biologics, medical devices, and cosmetics. The identification of carcinogens has depended classically upon two approaches, epidemiological studies and chronic animal bioassays, each of which has its own strengths and weaknesses. The development of new techniques to assess carcinogenic risk provides the basis for alternative methods of assessing carcinogenic potential that can augment, or perhaps even replace, the need for expensive chronic bioassays. The FDA benefits from these studies by having improved pre-clinical assessments that allow extrapolations across species and lead to more scientifically sound risk decisions for the public. The outcome of the Division's research is an integrated suite of technology for use in guiding pre-clinical assessments of compounds regulated by the FDA.

ACTIVE PROJECTS FY1999

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0210601	**Chronic Tumor Study of Fumonisin B_1 in Male and Female $B_6C_3F_1$ Mice** To determine if dietary fumonisin B_1 is tumorigenic to male and female $B_6C_3F_1$ mice following chronic dietary exposure.	Howard, Paul* Lorentzen, Ronald Voss, Kenneth A.
E0210611	**ADDEND: Chronic Tumor Study of Fumonisin B_1 in Male and Female $B_6C_3F_1$ Mice** To amend study director's name and change requirements for pathology. Also extending project to 1/14/99.	Howard, Paul* Lorentzen, Ronald
E0210801	**Chronic Tumor Study of Fumonisin B_1 in Male and Female F344 Rats** To determine the tumorigenicity of fumonisin B_1 in male and female F344 rats following chronic dietary exposure.	Howard, Paul* Lorentzen, Ronald Voss, Kenneth A.
E0210811	**ADDEND: Chronic Tumor Study of Fumonosin B_1 in Male and Female F344 RATS** Addendum submitted to change study director and change requirements of pathology - also extending project to 1/14/99.	Howard, Paul* Lorentzen, Ronald
E0211101	**The Role of Fumonisin B_1 in *Fusarium sp.* Tumorigenicity in Rats** Determine the effect of fumonisin B_1 on signal transduction pathways in cultured human esophageal epithelial tissues. Determine if DNA damage occurs *in vivo* in F344 rats when fed in the diet cultures of *Fusarium graminearum*, *Fusarium subglutinans, Fusarium moniliforme* or a combination of the three fungi, using 32P-postlabeling tech. Determine the pharmacokinetics of fumonisin B_1 in $B_6C_3F_1$ mice and F344 rats under conditions similiar to those used in the chronic bioassay, and in non-human primates.	Howard, Paul* Couch, Letha H. Melchior, William B. Slikker, William Sutherland, John B. Tolleson, William H. Miller, David Pohland, Albert
E0211111	**ADDEND: The Role of Fumonisin B_1 and other Mycotoxins *in Fusarium sp.* Tumorigenicity in Rats: Transplacental Pharmacokinetics of Fumonisin B_1 in the Rhesus Monkeys** Use the rhesus monkey as a model to determine if fumonisin B_1 crosses the placenta.	Howard, Paul* Binienda, Zbigniew K. Martinez, Marilyn Slikker, William
E0211121	**ADDEND: The Role of Fumonisin B_1 and Other Mycotoxins in *Fusarium sp.* Tumorigenicity in Rats** Addendum submitted due to recent data from the subchronic toxicity studies with fumonisin B_1 (E02113), and other results from Biochem Tox laboratory. Changes require additional use of animals over the needs described in the original protocol (E02111.01). 1. Training in orbital bleeding techniques; 2. Identification of rhesus monkeys; 3. Addition of new isolate of *Fusarium moniliforme* to list of fungi; 4. Use of primary cultures of mouse liver hepatocytes to investigate hepatotoxicity of fumonisin B_1; 5. Addition of female $B_6C_3F_1$ mice to pharmacokinetic studies; 6. Additional mice for Exp. 1 in E02111.01.	Howard, Paul* Casciano, Daniel A. Martinez, Marilyn Shaddock, Joseph G.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0211131	**ADDEND: The Role of Fumonisin B_1 and Other Mycotoxins in *Fusarium sp.* Tumorigenicity in Rats: Internal Deposition of [14C]Fumonisin B_1 in the Female Rhesus Monkey** To verify observation that the majority of [14C]Fumonisin B_1 administered to an overnight fasted rhesus monkey remains in the animal; requesting 1 rhesus monkey.	Howard, Paul* Binienda, Zbigniew K. Martinez, Marilyn Slikker, William
E0211141	**ADDEND: The Role of Fumonisin B_1 and other Mycotoxins in *Fusarium sp.* Tumorigenicity in Rats: Pathology Support for *In Vitro* Studies with Cultured Human Cells** To confirm and extend our observation that fumonisin B_1 induces apoptosis. Also, to request Pathology support be added to the protocol.	Howard, Paul* Martinez, Marilyn
E0211601	**Tumorigenicity of Chloral Hydrate in $B_6C_3F_1$ Mice** To determine the effect of animal age and duration of exposure upon the tumorigenicity of chloral hydrate in female $B_6C_3F_1$ mice.	Beland, Frederick A.* Benson, Robert W. Contrera, Joseph Gaylor, David W.
E0211901	**Development of Analytical Methods for Determination of Malachite Green** 1. Develop analytical methods to assess purity of malachite green (MG) and leuco-malachite green (LMG) that will be used in the NTP animal bioassay; 2. Develop analytical methods to quantify MG and LMG content and determine homogeniety and stability in rodent chow under storage and use conditions.	Doerge, Daniel R.* Churchwell, Mona I. Rushing, Larry G. Schmitt, Thomas C.
E0211911	**ADDEND: Development of Analytical Methods for Determination of Malachite Green and Anti-Thyroid Effects** To use the LC/MS methodology previously developed for determination of MG and LMG purity to: 1. Study the thyroidal and hepatic metabolism of MG and LMG; 2. Determine the mechanism for inhibition of thyroid hormone synthesis and induction of thyroid tumors observed for gentian violet (GV); 3. Determine if that mechanism is applicable to MG and use findings to clarify risks to humans using the upcoming NTP bioassay results. No increase in allotted time or funding is anticipated.	Doerge, Daniel R.* Churchwell, Mona I.
E0212001	**Effect of Ethanol on the Tumorigenicity of Urethane (Ethyl Carbamate) in $B_6C_3F_1$ Mice** To determine the effect of ethanol on the tumorigenicity of urethane (ethyl carbamate) in $B_6C_3F_1$ mice.	Beland, Frederick A.* Benson, Robert W. Chan, Po C. Lorentzen, Ronald Roberts, Dean W.
E0212101	**Development of Analytical Methods for Determination of Urethane** To develop analytical methods to assess purity and stability of urethane and ethanol that will be used as test compounds in the NTP rodent bioassay; Develop analytical methods to quantify urethane and ethanol content in aqueous dosing solutions and determine stability under storage and use conditions for the NTP bioassay; Develop analytical procedures to quantify the content of urethane in rodent feed.	Doerge, Daniel R.*

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0212201	**Range Finding Study for the Evaluation of the Toxicity of Genistein Administered in the Feed to CD (Sprague-Dawley) Rats (Without Behavioral Breeding)** To determine the doses of genistein to be used in a multigeneration bioassay for establishing the effects of this naturally occurring isoflavone on development of reproductive organs, reproduction, cancer of the reproductive organs, and neurological and immunological function.	Delclos, Kenneth B.* Newbold, Retha Weis, Constance C.
E0212211	**ADDEND: Range-finding Study (Genistein) – Request for addn'l animals for assessment of micronuclei in bone marrrow** Requesting additional animals in order to include an additional dose of mitomycin C to assess the potential induction of micronuclei in bone marrow of rats treated with genistein.	Delclos, Kenneth B.*
E0212214	**ADDEND: Evaluation of the Potential Effects of Genistein on the Immune System** The NTP has requested that data on the potential immunological effects of the test agents be collected during the range finding portion of the study. The conduct of this work will require animals in addition to those previously requested under E02122.01.	Delclos, Kenneth B.* Germolec, Dori Newbold, Retha Weis, Constance C.
E0212221	**ADDEND: Range Finding Study for the Evaluation of the Effects of Genistein Administered in the Feed to CD (Sprague-Dawley) Rats during Development** To clarify the pathology data requirements. Specifics for handling of certain tissus were not included in the original protocol or the Pathology Protocol: 1. Ovarian follicle counts; 2. Mammary whole mounts; 3. Femur measurements.	Delclos, Kenneth B.*
E0212301	**Range Finding Study for the Evaluation of the Toxicity of Methoxychlor Administered Feed to CD (Sprague-Dawley) Rats** To determine the doses of methoxychlor for use in a multigeneration bioassay for assessing the effects of this pesticide on the development of the reproductive tract, reproduction, cancer of the reproductive organs, and neurological and immunological function.	Delclos, Kenneth B.* Newbold, Retha Weis, Constance C.
E0212311	**ADDEND: Range-finding Study (Methoxychlor) - Addn'l animals for assessment of micronuclei in bone marrow.** Requesting additional animals in order to include an additional dose of mitomycin C in order to assess the potential induction of micronuclei in bone marrow of rats treated with methoxychlor.	Delclos, Kenneth B.*
E0212314	**ADDEND: Evaluation of the Potential of Methoxychlor on the Immune System (Immuno Component)** The NTP has requested that data on the potential immunological effects of the test agents be collected during the range finding portion of the study. The conduct of this work will require animals in addition to those previously requested under E02123.01.	Delclos, Kenneth B.* Germolec, Dori Newbold, Retha Weis, Constance C.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0212321	**ADDEND: Range Finding Study for the Evaluation of the Effects of Methoxychlor Administered in the Feed to CD (Sprague-Dawley) Rats during Development** To clarify the pathology data requirements. Specifics for the handling of certain tissues were not included in the original protocol or the Pathology Protocol on the following: 1) Ovarian follicle; 2) Mammary whole mounts; and 3) Femur measurements.	Delclos, Kenneth B.*
E0212322	**ADDEND: Range Finding Study for the Evaluation of the Effects of Methoxychlor Administered in the Feed to CD (Sprague-Dawley) Rats during Development** To clarify the pathology data requirements for Test 2 of E0212301. Specifics for the handling of certain tissues were not included in the original protocol or the Pathology Protocol on the following: 1. Ovarian follicle; 2. Mammary whole mounts; and 3. Femur measurement. Also, request for ext. until 6/99.	Delclos, Kenneth B.*
E0212401	**Comparative Toxicity of Fumonisin Derivatives in Female $B_6C_3F_1$ Mice** To compare the toxicity of several fumonisin derivatives in female $B_6C_3F_1$ mice.	Howard, Paul* Bucci, Thomas J. Couch, Letha H. Doerge, Daniel R. Pohland, Albert
E0212501	**Range Finding Study for the Evaluation of the Toxicity of Nonylphenol Administered in the Feed to CD (Sprague-Dawley) Rats** To determine the doses of nonylphenol for use in a multigeneration bioassay for assessing the effects of this compound on the development of the reproductive tract, reproduction, and neurological and immunological function.	Delclos, Kenneth B.* Newbold, Retha Weis, Constance C.
E0212511	**ADDEND: Range Finding Study for the Evaluation of the Effects of Nonylphenol Administered in the Feed to CD (Sprague-Dawley) Rats during Development** To clarify the pathology data requirements. Specifics for the handling of certain tissues were not included in the original protocol or the Pathology Protocol: 1. Ovarian follicle counts; 2. Mammary whole mounts; and 3. Femur measurements.	Delclos, Kenneth B.*
E0212514	**ADDEND: Evaluation of the Potential Effects of Nonylphenol on the Immune System** The NTP has requested that data on the potential immunological effects of the test agents be collected during the rangefinding portion of the study. The conduct of this work will require animals in addition to those previously requested under E02125.01.	Delclos, Kenneth B.* Germolec, Dori Newbold, Retha Weis, Constance C.
E0212601	**Range Finding Study for the Evaluation of the Toxicity of Vinclozolin Administered in the Feed to CD (Sprague-Dawley) Rats** To determine the doses of vinclozolin for use in a multigeneration bioassay for assessing the effects of this compound on the development of the reproductive tract, reproduction, and neurological and immunological function.	Delclos, Kenneth B.* Newbold, Retha Weis, Constance C.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0212611	**ADDEND: Range Finding Study for the Evaluation of the Effects of Vinclozolin Administered in the Feed to CD (Sprague-Dawley) Rats during Development** To clarify the pathology data requirements. Specifics for the handling of certain tissues were not included in the original protocol or the Pathology Protocol: 1) Ovarian follicle counts; 2) Mammary whole mounts; and 3) Femur measurements.	Delclos, Kenneth B.*
E0212614	**ADDEND: Evaluation of the Potential Effects of vinclozolin on the Immune System** The NTP has requested that data on the potential immunological effects of the test agents be collected during the range finding portion of the study. The results of these studies will determine the immunotoxicological endpoints to be evaluated in the multigeneration studies.	Delclos, Kenneth B.* Germolec, Dori Newbold, Retha Weis, Constance C.
E0212701	**Two-Year Bioassay in Mice Administered Malachite Green or Leucomalachite Green in the Diet** To determine the risk associated with exposure to malachite green or leucomalachite green.	Culp, Sandra J.* Beland, Frederick A. Benson, Robert W. Mulligan, Louis T.
E0212801	**Two-year Bioassay in Rats Administered Malachite Green or Leucomalachite Green in the Diet** To determine the risk associated with exposure to malachite green or leucomalachite green.	Culp, Sandra J.* Beland, Frederick A. Benson, Robert W. Mulligan, Louis T.
E0212811	**ADDEND: Two-Year Bioassay in Rats Administered Malachite Green or Leucomalachite Green in the Diet** Determine if the demethylated derivatives of malachite or leucomalachite green found in tissues of treated rodents lead to the reactive species that bind to DNA.	Culp, Sandra J.* Beland, Frederick A. Mulligan, Louis T.
E0212901	**Range Finding Study for the Evaluation of the Effects of Ethinyl Estradiol Administered in the Feed to CD (Sprague-Dawley) Rats During Development** To determine the doses of ethinyl estradiol (EE2) for use in a multigeneration bioassay for assessing the effects of this compound on the development of the reproductive tract, reproduction, and neurological and immunological function.	Delclos, Kenneth B.*
E0212911	**ADDEND: Range Finding Study for the Evaluation of the Effects of Ethinyl Estradiol Administered in the Feed to CD (Sprague-Dawley) Rats during Development** To clarify the pathology data requirements. Specifics for the handling of certain tissues were not included in the original protocol or the Pathology Protocol: 1) Ovarian follicle counts; 2) Mammary whole mounts; and 3) Femur measurements.	Delclos, Kenneth B.*
E0212914	**ADDEND: Evaluation of the Potential Effects of Ethinyl Estradiol on the Immune System** The NTP has requested that data on the potential immunological effects of the test agents be collected during the range finding portion of the study. The results of these studies will determine the immunotoxicological endpoints to be evaluated in the multigeneration studies.	Delclos, Kenneth B.* Germolec, Dori Newbold, Retha Weis, Constance C.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0213011	**ADDEND: A Comparison of Weight Gain and Fertility in CD Rats Fed a Standard Diet (NIH-31) or a Soy- and Alfalfa-free, Casein-containing Diet (NIH-31C)** To address basic questions as to the performance of the diet that has been selected for used in the endocrine disruptor multigeneration studies that will be carried out under the NCTR/NTP Interagency Agreement. In particular, questions of fertility, which is important to all aspects of all of the studies, and weight gain, which may be critically important in the chronic phase of the assessment of two of the compounds, are being addressed.	Delclos, Kenneth B.*
E0213101	**The Effects of Chemoexfoliation using α- and β-hydroxy Acids on Cell Proliferation and DNA Adduct Formation in SKH-1 Mice Exposed to Simulated Solar Light** The NIEHS/FDA Phototoxicity Center is designed to address the effects of compounds on the induction of skin cancer in mice using light sources that are relevant to humans. Input into the design of the facility has been obtained from experts in phototoxicity and photocarcinogenicity. These experts will continue to provide critical advice on the design of the experimental protocols. As a result, a facility will be developed that will meet the rigors of scientific scrutiny, and will generate data for human health risks from the effects of compounds on light induced skin cancer. The facility is also designed for expansion to allow simultaneous examination of the toxicity or cocarcinogenicity of compounds in the presence of either simulated sunlight or fluorescent UVB light. The mechanistic studies in this proposal will provide the data necessary to design and interpret properly the future α-hydroxy acid and simulated solar light cocarcinogenicity studies.	Howard, Paul* Beer, Janusz Miller, Barbara J. Wamer, Wayne
E0213201	**Genistein: Evaluation of Reproductive Effects Over Multiple Generations and the Chronic Effects of Exposure during Various Life Stages** 1. To determine the effects of genistein, a naturally occuring isoflavone, on reproduction and on the development of reproductive and selected other hormone-sensitive organs when administered to CD rats over multiple generations; 2. To determine if subtle effects observed in the dose range finding study are magnified through multiple generations; 3. To evaluate the reversibility of any observed effects; and 4. To evaluate the chronic toxicity of genistein, particularly potential induction of cancer of the reproductive organs, following exposures that will include various life stages (*in utero* through early adulthood, *in utero* and continuous for 2 years after birth, *in utero* and lactational only, and postweaning only).	Delclos, Kenneth B.* Newbold, Retha Weis, Constance C.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0213211	**ADDEND: Evaluation of Plasma Genistein Levels, Responses to Estrogen Challenge, and Alterations in Pathways of Sex Steroid Synthesis, Metabolism, and Response in Sprague-Dawley Rats Exposed Through Multiple Generations to Dietary Genistein** In the parent protocol, NCTR E02132.01, CD rats will be exposed to dietary genistein over multiple generations and effects on structure and function of the reproductive system and other estrogen-sensitive target organs will be evaluated. The purpose of the experiments described in this addendum is to utilize excess animals from the parent experiment to assess plasma and tissue genistein levels and evaluate additional endpoints modulated by genistein. These additional endpoints may be mechanistically associated with compound-induced alterations observed in the dose range finding studies that are also anticipated in animals from the multigeneration studies.	Delclos, Kenneth B.* Blaydes, Betty J. Branham, William S. Dalu, Abraham N. Doerge, Daniel R. Laurenzana, Elizabeth M. Newbold, Retha Sheehan, Daniel M. Weis, Constance C.
E0213221	**ADDEND: Evaluation of the Effects of Ethinyl Estradiol on the Estrogen Receptor Levels in Reproductive Tract and Mammary Gland: Comparison with the Effects of Genistein** Requesting that PAI use tissues already collected under E02129.14 to embed the fixed tissues and prepare slides to be utilized for immunohistochemistry. These tissues will be used for comparison with tissues to be evaluated under E02132.11. Additional pathology hours will be required.	Delclos, Kenneth B.*
E0213301	**A Study of Genotoxic and Secondary Mechanisms of Riddelliine Carcinogenesis** 1. To study the mechanisms of direct-acting genotoxicity (involving exogenous DNA adduct formation) of riddelliine; 2. To analyze riddelliine-derived DNA adducts in target tissues of rats treated with riddelliine as part of the NTP and chronic study, and from male anf female rats to be treated at the NCTR for a shorter period of time with riddelliine and its reactive metabolite, dehydroriddeliine; 3. If a dehydroretronecine-modified DNA adduct is detected in the liver tissues of animals treated with riddelliine, propose to determine whether or not this DNA adduct is also formed in animals treated with other tumorigenic pyrrolizidine alkaloids; 4. To compare the metabolic activation pathways and DNA adduct formation of the tumorigenic pyrrolizidine alkaloid, riddelliine, retrorsine, and monocrotaline, and a non-tumorigenic pyrrolizidine alkaloid, retronesine in rat and human liver microsomal systems.	Chou, Ming W.* Beger, Richard Chan, Po C. Doerge, Daniel R. Fu, Peter P. Nichols, Jasyl A. Von Tungeln, Linda S. Yang, Ya-chen
E0213501	**Para-Nonylphenol: Evaluation of Reproductive Effects over Multiple Generations** 1. Determine the effects of p-nonylphenol, an intermediate in the production of surfactants and other industrial products, on reproduction and on the development of reproductive and selected other hormone-sensitive organs when administered to CD rats over multiple generations; 2. Determine if subtle effects observed in the dose range finding study are magnified through multiple generations; 3. Evaluate the reversibility of any observed effects.	Delclos, Kenneth B.* Newbold, Retha Weis, Constance C.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0213511	**ADDEND: Evaluation of the Effects of Nonylphenol on Pathways of Sex Steroid Synthesis, Metabolism, and Response in Male CD (Sprague-Dawley) Rats** Utilize excess animals from the parent experiment to conduct mechanistic studies designed to address results from the p-nonylphenol range finding study that are also expected in rats from the multigeneration. Assess the effect of nonylphenol on aromatase activity in the central nervous system in male rats at birth and on testosterone production and steroid receptor expression at puberty and at postnatal day (PND) 140.	Laurenzana, Elizabeth M.* Blaydes, Betty J. Delclos, Kenneth B. Meredith, John M. Newbold, Retha Weis, Constance C.
E0260301	**Caloric Restriction and Gene Expression in Agouti Mice** The total amount of fat and calories we consume in our diet is highly correlated with the occurrence of cancer in North America and other highly developed nations. The studies proposed would help us learn how calories modify the development of cancer in mice and the mechanism underlying cancer development in humans.	Wolff, George L.* Kaput, James A. Visek, Willard J. Collins, Jerry
E0260311	**ADDEND: Caloric Restriction and Gene Expression in Agouti Mice** To adjust protocol to conform to the standard operating procedure (SOP) used in NCTR caloric restriction studies; also specifies the use of trioctanoin instead of tricaprylin as the solvent for dimethylbenz(a)anthracene (DMBA) because NCTR has a large supply of trioctanoin left over from previous studies; addendum requesting addional animals - 48 yellow mice and 48 agouti mice.	Wolff, George L.* Collins, Jerry
E0260313	**ADDEND: Caloric Restriction and Gene Expression in Agouti Mice** To request 48 additional agouti (BALB/c x VY)F_1 female mice due to unexpectedly unfavorable segregation of yellow and agouti mice in the litters produced for the last allocation to E02603.01 causing a deficiency of 48 yellow females.	Wolff, George L.* Collins, Jerry
E0260321	**ADDEND: Caloric Restriction and Gene Expression in Agouti Mice** Addendum submitted to specify that two weeks before the schedule sacrifice dates for the 30, 60, 90 and 120 day interim sacrifice mice, ~0.25 ml blood to be drawn from a retroorbital sinus of each mouse for insulin and glucose assays – will involve 32 mice; data to be obtained under this addendum will enable correlation of relative insulin and glucose levels.	Wolff, George L.* Collins, Jerry
E0260331	**ADDEND: Caloric Restriction and Gene Expression in Agouti Mice** To delete determination of liver weight at the time of sacrifice of the mice – change in Pathology protocol.	Wolff, George L.*
E0260341	**ADDEND: T.O. #589 – Final Report Support** To cover additional support needed from ADP contractor to provide reports, data files, answers, and documentation for four test numbers. Also requesting extension of project.	Wolff, George L.*

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0687501	**Mechanisms of Diet-Induced DNA Damage with Methyl Donor Deficiency** Further the understanding of the mechanisms by which diet, as an environmental variable, can alter the susceptibility to cancer.	James, Sandra J.* Pohland, Albert
E0687511	**ADDEND: Antioxidant Induced Changes in Rat Liver DNA Methylation Status** Determine whether the antioxidant Butylated Hydroxytoluene (BHT) (ionol) influences DNA methylation in the liver of rats.	James, Sandra J.* Poirier, Lionel A. Wise, Carolyn K.
E0687521	**ADDEND: Mechanism of DNA Damage With Dietary Methyl Deficiency** Requesting addition of 20 p53+/-heterozygous mice and 20 background (C57Bl/6) control mice - no changes in animal treatment.	James, Sandra J.*
E0687531	**ADDEND: DNA Damage with Dietary Methyl Donor Deficiency** Requesting purchase of 60 athymic nude mice to be used for confirmation of *in vitro* cell transformation of Chinese hamster ovary (CHO) cell lines.	James, Sandra J.*
E0687541	**ADDEND: Mechanisms of Diet-Induced DNA Damage with Methyl Donor Deficiency** The gavage procedure under E0687501 for pinworm elimination induced over a 20% weight loss during the tumorigenic period. Because weight loss can interfere with tumor progression, this experiment will repeat to verify that the pinworm treatment did not contribute to our final results.	James, Sandra J.*
E0687901	**The Evaluation of Selected Benzodiazepine and Antihistamine Drugs in the Neonatal Mouse Tumorigenicity Bioassay and in Transgenic Human Lymphoblastoid Cells** 1. To determine if the neonatal mouse bioassay can be employed to evaluate the tumorigenic potential of therapeutic drugs. 2. To examine concurrently as positive controls the genotoxic carcinogens: 4-aminobiphenyl, benzo(a)pyrene, 6-nitrochrysene, and aflatoxin B_1 3. To study the metabolism and DNA adduct formation of benzodiazepine and antihistamine drugs by mouse and human liver microsomes to determine which if any cytochrome P450 is responsible for metabolic activation in mice and humans. 4. Transgenic human lymphoblastoid cell lines expressing appropriate CYP isozymes will also be employed to study the mutations and DNA binding of the subject drugs.	Fu, Peter P.* Casciano, Daniel A. Contrera, Joseph Kadlubar, Fred F.
E0687912	**ADDEND: The Evaluation of Selected Benodiazepine and Antihistamine Drugs in the Neonatal Mouse Tumorigenicity Bioassay and in Transgenic Human Lymphoblastoid Cells** To determine whether or not the $B_6C_3F_1$ neonatal mouse tumorigenicity bioassay is sensitive to chemical carcinogens that exert their tumorigenic activity by a secondary mechanism, we propose to use two sets of chemical carcinogens, of which we have knowledge of their putative carcinogenic mechanism, for further study and request that this protcol be extended for additional tests.	Fu, Peter P.* Casciano, Daniel A. Kadlubar, Fred F. Teitel, Candee H. Von Tungeln, Linda S.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0687913	**ADDEND: The Evaluation of Selected Benzodiazepines and Antihistamine Drugs in the Neonatal Mouse Tumorigenicity Bioassay and Transgenic Human Lymphoblastoid Cells** To allow the test animals to stay on experiment an additional year, thereby possibly increasing the treatment response. Will require no additional animals or treatment, but will require additional routine animal care (housing of animals), additional In-Life support, and change termination of the experiment and sacrifice.	Fu, Peter P.* Contrera, Joseph Von Tungeln, Linda S.
E0687914	**ADDEND: The Evaluation of Selected Benzodiazepines and Antihistamine Drugs in the Neonatal Mouse Tumorigenicity Bioassay and in Transgenic Human Lymphoblastoid Cells** 1) Modify the original protocol by replacing the test chemical triprolidine with methylphenidate hydrochloride (ritalin); 2) Add the following four test chemicals: 4-hydroxy-2-nonenal, malondialdehyde, crotonaldehyde, and acrolein; 3) Modify the original protocol by combining the dose range studies for year two and three into one dose range study to be performed in year two and adding animals necessary to perform dose range on 4-hydroxy-2-nonenal, malondialdehyde, crotonaldehyde, and acrolein.	Fu, Peter P.* Casciano, Daniel A. Contrera, Joseph Kadlubar, Fred F. Teitel, Candee H. Von Tungeln, Linda S.
E0687915	**ADDEND: The Evaluation of Selected Benzodiazepines and Antihistamine Drugs in the Neonatal Mouse Tumorigenicity Bioassay and in Transgenic Human Lymphoblastoid Cells** Animals in the low dose experiment E06879.05, received one half the total dose of those animals in E06879.04. Based on the results of the high dose experiment (E06879.04), it is anticipated there will not be an increased tumor incidence observed in any of the low dose experimental animals if sacrificed after one year on test. Therefore, we request an extension of E06879.05 to allow the test animals to stay on experiment an additional three months, thereby possibly increasing the treatment response. This request will require no additional animals or treatment. Will require additional routine animal care, additional In-Life support, and change termination of the experiment and sacrifice from the week of December 2, 1996 to the week of March 3, 1997.	Fu, Peter P.* Von Tungeln, Linda S.
E0687916	**ADDEND: The Evaluation of Selected Benzodiazepines and Antihistamine Drugs in the Neonatal Mouse Tumorigenicity Bioassay and in Transgenic Human Lymphoblastoid Cells** Add two test chemicals, phenolphthalein and Tacrin, to year three of the master protocol. These two commonly used drugs are now of immediate concern to FDA due to recent positive results in carcinogenicity and/or genotoxicity studies. Will be adding four treatment groups to the third year studies now in progress. No additional dams will be requested. Addendum involves additional housing and dosing costs.	Fu, Peter P.* Contrera, Joseph Kadlubar, Fred F. Teitel, Candee H. Von Tungeln, Linda S.
E0687917	**ADDEND: The Evaluation of Selected Benzodiazepine and Antihistamine Drugs in the Neonatal Mouse Tumorigenicity Bioassay and in Transgenic Human Lymphoblastoid Cells** To determine the tumorigenicity, mutagenicity, and DNA adduct formation of anti-HIV nucleosides in the $B_6C_3F_1$ neonatal mouse bioassay.	Beland, Frederick A.* Doerge, Daniel R. Fu, Peter P Heflich, Robert H. Poirier, Mimi C. Von Tungeln, Linda S.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0687918	**ADDEND: The Evaluation of Selected Benzodiazepine and Antihistamine Drugs in the Neonatal Mouse Tumorigenicity Bioassay and in Transgenic Human Lymphoblastoid Cells** Requesting that the reproductive organs of female mice on E06879.08 and E06879.09 be examined microscopically. In addition, the reproductive organs of the males should also be examined microscopically to determine if perturbations are induced. This request will not require additional animals or treatment, however, additional pathology expense will be accrued by processing and examination of additional tissues.	Fu, Peter P.* Von Tungeln, Linda S.
E0688201	**Tumor Promotion and Neurochemical Changes in Mice During Chronic Feeding of the Anti-depressant Fluoxetine** 1. To determine if chronic feeding of fluoxetine (Prozac) results in promotion of mouse mammary carcinomas; 2. To determine if chronic feeding of fluoxetine: a) produces changes in the concentrations of serotonin and its metabolite, 5-hydroxyindoleacetic acid, in different regions of the mouse brain; b) induces changes in serotonergic receptor and uptake sites in different regions of the mouse brain.	Wolff, George L.* Ali, Syed F. Contrera, Joseph
E0688211	**ADDEND: Tumor Promotion and Neurochemical Changes in Mice during Chronic Feeding of the Anti-depressant Fluoxetine** To change route of administration of drug from feed to water.	Wolff, George L.* Ali, Syed F. Contrera, Joseph
E0688801	**A Collaborative Research Proposal to Assess Cancer Risk Posed by Intermittent Exposure to Aflatoxin B_1 (AFB_1) in Rats** 1. To test the hypothesis that a chemically induced tumor incidence is a function of the accummulated lifetime exposure, and is predictable from the average daily dose for various dosing regimens, such as continous and intermittent dosing; 2. To study correlations between the chemically-induced tumor incidence and various biomarkers of the initiation and the promotion stage of carcinogenesis for continuous and intermittent dosing.	Chou, Ming W.* Aidoo, Anane Allaben, William T. Bowers, John Casciano, Daniel A. Gaylor, David W. Giri, Chandrakan P. Hinton, Dennis James, Sandra J. Kodell, Ralph L. Morris, Suzanne M. Roth, William Sahu, Saura Sotomayer, Rene Warbritton, Alan R.
E0688811	**ADDEND: A Collaborative Research Proposal to Assess Cancer Risk Posed by Intermittent Exposure to Aflatoxin B_1 in Rats** Animals needed for the preparation of conditioned media (CM) and autologous cells were inadvertently left out of the original animal request on the master project (E06888.01). This addendum requests 60 male F344 rats for this purpose.	Chou, Ming W.*

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0691201	**Cellular and Molecular Responses to Chronic Iron Overload in Animal Models** 1. To determine the health effects of chronic iron overload in mice and rats. 2. To determine neurochemical changes after chronic iron overload in mice and rats. 3. To develop an animal model for identifying the cellular and molecular mechanisms underlying the hepatic and pancreatic effects of chronic iron overload which are characteristic of the human disease idiopathic hemochromatosis and possible neurochemical mechanisms which associate effects of iron with neurological disorders, e.g., Parkinson and Alzheimer diseases.	Wolff, George L.* Ali, Syed F. Whittaker, Paul
E0691211	**ADDEND: Cellular and Molecular Responses to Chronic Iron Overload in Animal Models** To authorize several changes in protocol: 1. Regular drinking water will be used instead of deionized distilled water; 2. Serum insulin will be added to the blood analytes; 3. (Apo)ferritin will be added to the immunohistochemical assays; 4. Dr. James-Gaylor will assay DNA strand breaks in frozen liver samples and will analyze tumor tissue in histopath sections.	Wolff, George L.* Ali, Syed F. James, Sandra J. Whittaker, Paul
E0691221	**ADDEND: Cellular and Molecular Responses to Chronic Iron Overload in Animal Models** To modify master project: Two daily death checks (instead of only one) will be performed; change in sacrifice procedure.	Wolff, George L.* Ali, Syed F. Whittaker, Paul
E0692001	**Toxic Hazards from Anti-thyroid Chemicals** Determine inhibition mechanisms for environmental goitrogens using purified thyroid peroxidase and lactoperoxidase; Determine the mechanism for covalent binding suicide substrates to purified peroxidases using electrospray-mass spectrometry to analyze intact adducted proteins and/or proteolytic fragments; Determine mechanism of goitrogen uptake into isolated thyroid cells in primary culture and subsequent inhibition of iodination/coupling reactions involved in thyroid hormone synthesis; Determine the structure-activity relationship for uptake of goitrogens into the thyroid and inhibition of thyroid hormone synthesis rates.	Doerge, Daniel R.* Chang, Chwen-wen Churchwell, Mona I. Rao, Divi L. Alderson, Norris
E0692011	**ADDEND: Determination of Anti-Thyroid Properties of Dietary Genistein** 1. Correlate *in vivo* thyroid hormone status endpoints from the NTP endocrine disruption rat bioassay with the *in vitro* activity of rat microsomal thyroid peroxidase (TPO) to better define the anti-thyroid mechanism for genistein; 2. Determine whether administration of genistein to rats results in an increase in the amounts of circulating auto-antibodies to TPO; 3. Develop analytical methodology to quantify genistein/daidzein and the respective sulfate and glucuronide conjugates in blood and mammary tissue.	Doerge, Daniel R.* Chang, Chwen-wen Churchwell, Mona I. Holder, Claude L.
E0692501	**DNA Adduct Formation by Nicotine Metabolites** 1. Determine the structural identity of the nicotine delta 1',2'- and delta 1',5'-iminium ion DNA adducts, and modify existing 32P-post labelling techniques to detect the adduct; 2. Quantify the presence of these adducts *in vitro* and *in vivo* in mice.	Howard, Paul* Cashman, John R. Doerge, Daniel R.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0694501	**Development of Methods for Analysis and Confirmation of β-Agonists** 1. Develop determinative and confirmatory procedures using Liquid Chromatography-Atmospheric Pressure Chemical Ionization Mass Spectrometry (LC-APCI/MS) for multiresidue screening β-agonists in livestock tissues; 2) Develop synthetic procedures to produce authentic β-agonist standards for use in regulatory screening. These methods will provide the flexibility to adapt to the potential for "designer drug" modifications by clandestine laboratories; 3) Explore the use of packed column supercritical fluid chromatography (SFC) coupled to APCI/MS as a more efficient technique for chromatographic separation in the screening of large numbers of β-agonists in livestock tissue.	Doerge, Daniel R.* Holder, Claude L. Settepani, Joseph
E0695201	**Effects of Dietary Restriction on the Post-Initiation Stages in Aflatoxin B_1-Induced Carcinogenesis on Male F-344 Rats Fed Methyl-Deficient Diets** To study the interactions of dietary restriction (DR) and methyl deficiency (MD) on the alterations of hepatic oxidative DNA damages, DNA methylation, cell proliferation, oncogene and tumor suppressor gene mutation, preneoplastic foci formation and tumor incidence during the post-initiation stages of AFB_1-induced carcinogenesis in male F344 rats. The results of these studies will: (i) test the hypothesis that DR may be an antagonist to the promotional effect of MD in the AFB_1-induced carcinogenesis; and (ii) evaluate the correlations between the effects of DR and MD on the formation of AFB_1-induced preneoplastic foci and tumors and various biomarkers during the post-initiation stages of carcinogenesis.	Chou, Ming W.* Aidoo, Anane Jackson, Carlton D. James, Sandra J. Poirier, Lionel A. Pohland, Albert
E0696101	**Mechanisms of Immunotoxicity and Carcinogenicity Associated with Silicone Breast Implants** Examine the acute and chronic cellular and subcellular responses to sub-cutaneous silicone implants utilizing state-of-the-art immunohistochemistry and molecular biology technologies.	James, Sandra J.* Miller, Barbara J. Pogribna, Marta V. Kammula, Raju
E0696111	**ADDEND: Mechanisms of Immunotoxicity and Carcinogenicity Associated with Silicone Breast Implants** To request a species change from F344 rats to the TG.AC transgenic mouse strain. Changed animal requirements to 52 transgenic mice, 200 F344 rats (Phase 2), and 20 $B_6C_3F_1$ mice for methods development.	James, Sandra J.*
E0696301	**Sexual Dimorphism in the Inflammatory Response to Biomaterials** Determine if a sex difference in the *in vitro* response of human monocytes and mouse peritoneal macrophages to various biomaterials can be demonstrated. Based on existing literature, we hypothesize that there will be a significant sex difference in the synthesis and release of inflammatory mediators that could influence the biocompatibility of the material.	Delclos, Kenneth B.* Chen, Xiaoling Colvert, Maureen Eaton, Daniel Klimberg, Suzanne

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0696311	**ADDEND: Sexual Dimorphism in the Inflammatory Response to Biomaterials** Analysis of the samples collected from the initial study indicated that the goals of the protocol could be better met with a shift in the experimental design which will be accomplished by: 1. Increase the size of the groups from 4 to 6 to increase statistical power; 2. An equal number of sham-operated controls will be needed at each time point to be used in quantititative comparisons of cytokine levels; 3. Extend time of implantation (from 6 to 15 weeks).	Delclos, Kenneth B.*
E0696701	**Investigations into the Interactive Oxidative Effects of Magnesium and Calcium with Selected Heavy Metals** To evaluate the influence of magnesium and calcium, both alone and in combination, on the toxicity from selected heavy metals in respect to the induction of oxidative DNA damage; To investigate the occurrence of adaptive responses in respect to the occurrence of oxidant stress from heavy metal toxicity; To evaluate interactions of the anti-oxidant ascorbate in respect to oxidative damage from selected heavy metals; To gain insight into mechanisms of action in regard to the toxicity and tumorigenic process instigated by heavy metals.	Littlefield, Neil A.* Chou, Ming W. Hass, Bruce S. Mikhailova, Marina V.
E0697001	**Sequence Specificity of DNA Adduct Formation and Removal Following Chronic Carcinogen Administration** To determine whether or not certain nucleotide sequences bearing carcinogen adducts are more resistant than others to DNA adduct formation and repair, and to identify these sequences.	Smith, Beverly A.* Beland, Frederick A. Marques, Matilde M.
E0697011	**ADDEND: Sequence Specificity of DNA Adduct Formation and Removal Following Chronic Carcinogen Administration** To determine the number and types of mutations induced in spleen T-lymphocytes by 2-acetylaminofluorene, (2AAF) and to determine how these compare with the distribution of DNA adducts within spleen lymphocyte and liver DNA. We proposed to use 2-AAF adducts induced in rat liver as a model system, with ligation-mediated PCR (LMPCR) as the method of choice for selection and amplification of modified genomic DNA. In this addendum, we propose to investigate the numbers and types of mutations induced in spleen T-lymphcytes in the rats being fed 2-AAF. Requesting 45 additional animals to complete this objective and adding a CO-PI and Res Support personnel.	Smith, Beverly A.* Beland, Frederick A. Fullerton, Nancy F. Heflich, Robert H.
E0697021	**ADDEND: Sequence Specificity of DNA Adduct Formation and Removal Following Chronic Carcinogen Administration** Division has been using a method to prepare hepatic nuclei. While this gives high-molecular-weight DNA preparations suitable for DNA adduct analyses, they have determined that the DNA still has too many breaks for LMPCR. Have established that this problem can be circumvented by isolating DNA from whole liver rather than from nuclei. Requesting 40 additional animals to complete this objective.	Smith, Beverly A.*
E0698701	**Tandem Immunochemical - Analytical Methods** Develop combined immunochemical and analytical chemical techniques to clean up complex matrices containing analytes of regulatory interest and provide detection at low concentrations with selectivity capable of providing structural confirmation.	Roberts, Dean W.* Benson, Robert W. Doerge, Daniel R. Gehring, Theresa A. Newkirk, Donald K.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0699301	**Evaluation of Host Factors Contributing to Differences in the Response to Biomaterials** To examine model systems that may be useful in the study of factors that regulate the extent of, and adverse effects arising from, the response to foreign bodies. As oxidative stress, including oxidative DNA damage, may play a major role in the foreign body reaction and in certain long-term adverse effects that may be associated with that reaction, we will also evaluate the utility of the air pouch model of inflammation to study species and strain differences in the development of and response to oxidative DNA damage.	Delclos, Kenneth B.* Blaydes, Betty J. Chen, Xiaoling Sams, Reeder L.
E0699311	**ADDEND: Evaluation of Host Factors Contributing in Differences in the Response to Biomaterials** To change pathology requirements originally requested – dropping request for any Proliferating Cell Nuclear Antigen (PCNA) staining for this experiment – requesting Pathology Associates Inc. (PAI) to carry out the appropriate staining and analysis of sections in order to analyze a subset of the tissues for apoptosis.	Delclos, Kenneth B.*
E0700301	**Nutritional Modulation of Apoptosis and Chemosensitivity: A Novel Anticancer Strategy** 1. In Nitroso methylurea (NMU)-initiated mammary epithelial cells, to determine whether nutritional manipulation of the cell cycle combined with low dose chemotherapy will permanently eliminate p53-independent and p53-dependent preneoplastic and neoplastic cells; 2. To determine the mechanisms of cell death induced by nutritional manipulation and low dose chemotherapy by examining molecular endpoints associated with p53-dependent and independent pathways of apoptosis.	James, Sandra J.* Hart, Ronald W. Muskhelishvili, Levan Pogribny, Igor P.
E0700401	**A Study of the Secondary Mechanisms of Carcinogenesis: Lipid Peroxidiation and Endogenous DNA Adduct Formation from Chloral Hydrate, Benzodiazepines, Antihistamines, and Other Chemicals** The specific aims outlined below are critical for the development of methodologies to study secondary mechanisms of carcinogenesis, including lipid peroxidation and endogenous DNA adduct formation, for determination of the mechanisms by which chemicals, such as FDA regulated drugs including benzodiazepines and antihistamines, may induce cancer, and for the continued development of the neonatal mouse bioassay as a regulatory alternative tumorigenicity bioassay: 1. To develop analytical methodologies for analysis of lipid peroxidation products and endogenous DNA adducts; 2. To determine whether or not the drugs of FDA interest, including benzodiazepines and antihistamines studied in E687901, and other chemicals induce lipid peroxidation and endogenous DNA adduct formation *in vitro*; 3. To determine the inhibitory effect of lipid- and water-soluble antioxidants on drug-induced lipid peroxidation and endogenous DNA adduct formation *in vitro*; 4. To determine whether or not the malondialdehyde-modified MG-1 DNA adduct and/or other endogenous DNA adducts can be used as biomarkers of lipid peroxidation; 5. To determine the mutagenicity of the benzodiazepine and antihistamine drugs in *Salmonella typhimurium* TA 104 and determine whether or not mutagenicity in *Salmonella typhimurium* TA104 can be used as a biomarker of lipid peroxidation induced by chemicals that generate free radicals upon metabolism.	Fu, Peter P.* Von Tungeln, Linda S. Yi, Ping Yin, Jun-Jie

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0700411	**ADDEND: A Study of the Secondary Mechanisms of Carcinogenesis: Lipid Peroxidation and Endogenous DNA Adduct Formation from Chloral Hydrate, Benzodiazepines, Antihistamines, and other Chemicals** Breeders should have been requested in the original protocol to facilitate the pup requirement and prevent scheduling conflicts or unnecessary animal distress. Requesting to be allowed to produce own $B_6C_3F_1$ pups for this purpose in Bldg. 53. Can be accomplished by the addition of 42 female C57BL/6N and 14 male C3H/HeNMTV to the original protocol. Will require additional animal care due to the maintenance of these requested breeders.	Fu, Peter P.* Von Tungeln, Linda S. Yin, Jun-Jie Macgregor, Jim
E0701101	**DNA Adducts of Tamoxifen** The nonsteroidal antiestrogen tamoxifen, which is currently being used in clinical trials as a chemoprotective agent against breast cancer, has been associated with the induction of certain malignancies. In order to determine if tamoxifen is acting through a genotoxic mechanism, this project will characterize DNA adducts from suspected tamoxifen metabolites, and develop methods for their detection and quantitation.	Beland, Frederick A.* Marques, Matilde M.
E0701111	**ADDEND: DNA Adducts of Tamoxifen** As discussed in preliminary data, there appear to be two major DNA adducts formed in rat liver. One of these adducts appears to arise from a-hydroxy-tamoxifen, while the identity of the other is presently unknown. This addendum proposes that a metabolite of a-hydroxy-N-desmethyl-tamoxifen is responsible for the second major adduct detected in rats treated with tamoxifen. Requesting additional animals for this proposal.	Beland, Frederick A.* Gamboa da Costa, Goncalo Marques, Matilde M.
E0701121	**ADDEND: DNA Adducts of Tamoxifen** Propose to treat rats with additional compounds, which are now available. Requesting 32 additional animals for this portion of the study.	Beland, Frederick A.* Marques, Matilde M.
E0701201	**Molecular Basis of Tumor Promotion and Increased Somatic Growth in Yellow Avy/a Mice: Mitogenic Effects of Agouti Protein *In Vitro*** To determine whether or not the agouti protein stimulates mitogenesis *in vitro.*	Tolleson, William H.* Wolff, George L.
E0701601	**Molecular and Metabolic Determinants of Maternal Risk and Progression of Down Syndrome: Potential for Nutritional Interventions** 1. To define abnormalities in one-carbon metabolism in mitogen-stimulated lymphocytes from women who have had a child with Down Syndrome and to determine whether appropriate folate/methyl supplementation can normalize these metabolic abnormalities; 2. To define the biochemical and molecular consequences of abnormal one-carbon metabolism in mitogen-stimulated lymphocytes from Down Syndrome children and to determine whether these metabolic abnormalities can be normalized with targeted nutritional intervention.	James, Sandra J.* Ames, Bruce N. Gibson, James B. Hine, R. Jean

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0702301	**The Role of Human Metabolism in Endocrine Disruption** Humans may be exposed to compounds in the diet or in the environment that disrupt endogenous endocrine responses in various tissues. We propose to utilize cell biological approaches to determine the role of human cytochromes P-450, UDP-glucuronosyltransferases, and sulfotransferases in the antiestrogens. The relative abilities of the various human enzyme systems expressed by individual cell lines to alter the extent of green fluorescent protein synthesis will indicate those human enzyme activities that activate or deactivate endocrine disrupting agents.	Tolleson, William H.* Howard, Paul Jenkins, Ronald E. Leakey, Julian E. Morris, Suzanne M. Rowland, Kenneth L.
E0702701	**The Effects of Dietary Genistein on the Growth of Chemically-Induced Mammary Tumors in Ovariectomized and Intact Rats** To determine whether or not, in the absence of endogenous ovarian estrogens, dietary genistein can promote or suppress the growth of neoplastic mammary tissue at various stages for the carcinogenic process.	Dalu, Abraham N.* Delclos, Kenneth B.
E0702711	**ADDEND: The Effects of Dietary Genistein on the Growth of Chemically-Induced Mammary Tumors in Ovariectomized and Intact Rats** Additional assistance needed from pathology with sacrificing rats in order to to sacrifice rats within two-hours time frame on a given day. Need assistance of one technician from pathology. Also requesting routine histopathological evaluation of the ovaries to be performed by the study pathologist. Also requesting vaginal lavages from Bionetics personnel on the last 5 consecutive days before sacrificing rats.	Dalu, Abraham N.*
E0705701	**Antigenic Biomarkers of Estrogen Catechol Metabolism for Use in Epidemiological Studies** 1. To prepare immunogenic conjugates for immunization of rabbits and antigenic conjugates for the characterization of antisera and for affinity purification of antibodies; 2. To develop IA/LC/MS methods to detect the antigenic biomarkers in urine and/or serum; 3. To initiate studies to validate the use of the antibody reagents and IA/LC/MS methods developed in Aim 1 and 2 using human urine and serum samples collected in an ongoing study of reproductive events, carcinogen metabolism, and interindividual variability.	Roberts, Dean W.* Doerge, Daniel R. Thompson-carino, Patricia
E0705901	**Purification of Ceramide Synthase** 1. Isolate rat ceramide synthase; 2. Identify the gene coding for rat ceramide synthase; 3. Develop antibodies to rat ceramide synthase; 4. Use the antibodies to study tissue specific expression of ceramide synthase.	Howard, Paul* Couch, Letha H. Jenkins, Ronald E. Melchior, William B. Roberts, Dean W. Tolleson, William H.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
P00392	**Development of Analytical Methods for Ethinylestradiol in Rodent Feed** The hypothesis that environmental chemicals with estrogenic activity cause reproductive problems and cancer of the reproductive tract in humans is based in part on the adverse outcomes observed in wildlife and the known effects of diethylstilbestrol in humans. The potential for reproductive and developmental toxicity of environmental chemicals are the focus of the Endocrine Disrupter Study of the National Toxicology Program in conjunction with the National Center for Toxicological Research. As part of this study, the oral contraceptive agent ethinylestradiol will be tested by lifelong feeding to rats and following the animals through multiple generations for adverse effects, including carcinogenesis. Central to these studies is the ability to quantify the content of ethinylestradiol in dosing medium. Because of the high potency of ethinylestradiol, the challenge of this project will be coupling a low dosing level with the complex suite of coextractive compounds found in rodent feed.	Doerge, Daniel R.* Holder, Claude L. Siitonen, Paul H.
P00402	**Procedures with SKH-1 Hairless Mice** 1. Personnel need to be trained in the proper use of the AIMS tattoo system. 2. Several personnel need to be trained in the administration of bromodeoxyuridine (BrdU) solutions to mice, and extraction of skin and duodenum tissue following sacrifice. 3. The method for the application of alpha-hydroxy acid containing cream to the skin of the mice needs to be determined, and SOPs developed. 4. The methods that will be used in processing the skin tissues from the hairless mice are not currently covered in a SOP.	Howard, Paul*
P00405	**Identification of the CEBLS Toxin in Lake DeGray** 1. Determine if a correlation exists between vacuolar myelinopathy in the coots and toxicity of coot organ extracts following injection into mice; 2. Isolate and identify the causative agent; 3. Establish a method for toxin detection; 4. Provide interested investigators (e.g., NCTR Division of Neurotoxicology) with enriched or purified toxin for determination of the mechanism of action of toxicity in rodents or birds.	Howard, Paul* Couch, Letha H. Doerge, Daniel R. Melchior, William B. Scallet, Andrew C.

PROJECTS COMPLETED FY1999

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0667800	**Neonatal Mouse Bioassay of Eight Complex Mixtures and Three Positive Control Samples** 1. To use neonatal male $B_6C_3F_1$ mice to determine the tumorigenic activity of eight complex mixture samples (i.e., smoky coal, aluminum smelter emissions, ambient air, cigarette smoke, coke oven emissions, diesel exhaust, polyethylene incineration and roofing tar), three positive control samples (i.e., benzo(a)pyrene, 6-nitrochrysene, and 4-aminobiphenyl) and their carrier (DMSO). 2. To remove target tissues from treated animals and send to EPA for carcinogen-DNA adduct quantitation and characterization. 3. To prepare appropriate synthetic standards for carcinogen-DNA adduct detection.	Fu, Peter P. * Dooley, Kenneth L. Kadlubar, Fred F.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0667820	**Mouse Skin Tumor Initiating Activity of Two Ambient Air Samples** Use female Sencar mice to determine the skin tumor initiating activity of two organic extracts of ambient air particulate matter, provided by EPA.	Fu, Peter P. * Dooley, Kenneth L. Kadlubar, Fred F.
E0684800	**The Effect of Dietary Magnesium on the Induction of Tumors, Transformation of Cells, and Leukemia Incidence** 1. To identify and evaluate the appearance of tumors, cell transformation, disruption of cell cycles and increased incidences of leukemia that may be related to dietary Mg deficiency, and 2. evaluate possible modulations of tumor expression through possible interactions of Mg and a carcinogenic metal, such as Ni.	Littlefield, Neil A. * Hass, Bruce S. Poirier, Lionel A.
E0696401	**Prevention of Ubiquitous Synthesis of the Agouti Protein by Methyl Supplemented Diet** To test the hypotheses that dietary methyl supplements fed to pregnant mice: 1. Increase expression of the pseudoagouti phenotype and decrease expression of the yellow phenotype among Avy/a offspring; 2. have no adverse gross morphological effects on the offspring 3. Increase the proportion of methylated cytosines in IAP promoter sequences in Avy/a offspring; and 4. Affect global DNA methylation levels and methyl metabolism in the dams and fetuses, as well as postnatal DNA metylation of the offspring.	James, Sandra J. *
E0696411	**ADDEND: Prevention of Ubiquitous Synthesis of the Agouti Protein by Methyl Supplemented Diet** Detection of nonpathogenic mode (Penicillium) at unacceptable levels in an earlier step of diet processing necessitates monitoring of the repelleted feed for bacteria and mold by Microbiology before it is fed to the animals.	Wolff, George L. * Cooney, Craig A.
E0696431	**ADDEND: Prevention of Ubiquitous Synthesis of the Agouti Protein by Methyl Supplemented Diet** The previous diet change has resulted in the production of litters each of which contain "almost pseudoagouti" mice with a very few small pale yellow stripes and spots. In order to establish whether the combined proportion of pseudoagouti + almost pseudoagouti offspring from dams receiving the new diet is statistically greater than expected, we will need a maximum of 20 additional litters each from Strains 29 and 46.	Wolff, George L. * Cooney, Craig A.

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2. Beland, F.A., Doerge, D.R., Churchwell, M.I., Poirier, M.C., Schoket, B., and Marques, M.M. Synthesis, characterization, and quantitation of a 4-aminobiphenyl DNA adduct standard. Chemical Research in Toxicology, 12:68-77, 1999. Accepted: 11/4/99. **(S00198)**.

3. Beland, F.A., Hamilton, L.P., and Marques, M.M. Comparison of the DNA adducts formed by tamoxifen and 4-hydroxytamoxifen *in vivo*. Carcinogenesis, 20:471-477, 1999. Accepted: 11/4/98. **(E0701101)**.

4. Chae, Y.H., Thomas, T., Guengerich, F.P., Fu, P.P., and El-Bayoumy, K. Comparative metabolism of 1-,2-, and 4-nitropyrene by human hepatic and pulmonary microsomes. Cancer Research, 59:1473-1480, 1999. Accepted: 2/2/99. **(E0687901)**.

5. Chae, Y.H., Ji, B. Lin, J., Fu, P.P., Cho, B.P., and El-Bayoumy, K. Nitroreduction of 4-nitropyrene is primarily responsible for DNA adducts formation in the mammary gland of female CD rats. Carcinogenesis, 12:180-186, 1999. Accepted 2/2/99. **(E06879.01)**.

6. Chang, C., Holland, R.D., Churchwell, M.I., and Doerge, D.R. Inactivation of *Coprinus cinereus* peroxidase by 4-chloroaniline during turnover: Comparison with horseradish peroxidase and bovine lactoperoxidase. Chemico-Biological Interactions, 123:197-217, 1999. Accepted: 8/15/99. **(E06920.01)**

7. Culp, S.J., Blankenship, L., Kusewitt, D.F., Doerge, D.R., Mulligan, L.T., and Beland, F.A. Toxicity and metabolism of malachite green and leucomalachite green during short-term feeding to Fischer 344 rats and $B_6C_3F_1$ mice. Chemico-Biological Interactions, 122:153-170, 1999. Accepted: 6/4/99. **(E0211801)**

8. Fu, P.P. and Herreno-Saenz, D. Nitro-polycyclic aromatic hydrocarbons: A class of genotoxic environmental pollutants. Environ. Carcinogen. Ecotoxicol. Rev., CIT(1): 1-43, 1999. Accepted: 3/25/99. **(E06879.01)**

9. Fu, P.P., Von Tungeln, L.S., Chiu, L., and Own, Z.Y. Halogenated-polycyclic aromatic hydrocarbons: A class of genotoxic environmental pollutants. Environ. Carcino. and Ecotox. Revs., C17(2): 71-109, 1999. Accepted: 5/12/99. **(E07004.01)**.

10. Gaylor, D.W., Culp, S.J., Goldstein, L., and Beland, F.A. Cancer risk estimation for mixtures of coal tars and benzo(a)pyrene. Risk Analysis, Accepted: 4/20/99. **(Collaborating with Ofc. of Dir./Imm. Ofc.) (E0672202)**

** (_ _ _ _ _ _ _) Indicates the related NCTR project number; NA-Not Applicable*

11. Hansen, D.K., Young, J.F., Laborde, J.B., Wall, K.S., and Holson, B. Pharmacokinetic considerations of dexamethasone-induced developmental toxicity in rats. J. Toxicological Sciences, 48:230-239, 1999. Accepted: 5/21/99. **(Collaborating with Gen & Repro. Tox.) (E0663812)**

12. Hart, R.W., Bucci, T.J., Turturro, A., Leakey, J.E., Feuers, R.J., Duffy, P.H., James, S.J., Lyn-Cook, B.A., Pipkin, J.L. and Li, S. Caloric intake as a modulator of carcinogenicity and anticarcinogenicity. In: *Carcinogenic/Anticarcinogenic Factors in Food: Novel Concepts*, Accepted: 3/12/99. **(Collaborating with Ofc. of Dep. Dir.) (E0260112)**

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15. Holder, C.L., Churchwell, M.I., Doerge, D.R. Quantification of genistein and metabolites in rat blood using LC-ES/MS J. Agricultural and Food Chemistry, 47:3764-3770, 1999. Accepted: 7/12/99. **(E0213201)**

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19. Mourato, L.M., Beland, F.A., and Marques, M.M. ^{32}P-Postlabeling of N-(deoxyguanosin-8-yl)arylamine adducts. A comparative study of labeling efficiencies. Chemical Research in Toxicology, 12: 661-669, 1999. Accepted: 5/24/99**. (E0697001)**

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NEUROTOXICOLOGY

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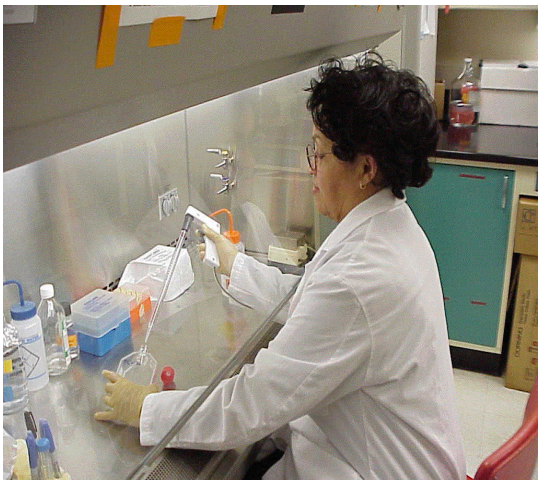

Cell culture facility in the Division of Neurotoxicology used to aid in the identification of biomarkers and mechanisms of neurotoxicity.

INTRODUCTION

In the United States, brain-related disorders account for more hospitalizations than any other major disease group, including cancer or cardiovascular diseases. One out of four Americans will suffer from a brain-related disorder at some point in their life, and the estimated annual cost to the national economy for treatment, rehabilitation and related consequences is $400 billion. At no time in the past, however, have researchers been better poised to increase understanding of brain-related disorders and to reduce the risks associated with neurotoxicity.

According to the Congressional Office of Technology Assessment's report, "Neurotoxicity: Identifying and Controlling Poisons of the Nervous System," (April 1990) the known or suspected causes of brain-related disorders include exposures to chemicals such as therapeutic drugs, food additives, foods, cosmetic ingredients, pesticides and naturally occurring substances. The number of potential neurotoxicants that require FDA regulation is estimated to be in the thousands and yet guidelines for neurotoxicity risk assessment remain vague and underdeveloped compared to those for cancer. Chemicals such as those listed above are vital to the national economy and our daily lives are markedly improved by them. The challenge is to determine at what dose and under what conditions a specific chemical may produce nervous system-related toxicity.

FY1999 ACCOMPLISHMENTS

The interdisciplinary approach, the use of multiple, established animal models and innovative biomarkers, and an in-depth working knowledge of and experience with mechanistically based focal areas of research, enable the neurotoxicology research group to be extraordinarily responsive to FDA regulatory needs. Several ongoing or planned studies, many in conjunction with other FDA centers, exemplify the application of the group's approach to providing critical research information applicable to FDA's regulatory problems. For example, the seafood neurotoxicant, domoic acid, and the prototypical excitotoxicant, kainic acid, are being evaluated in conjunction with colleagues at the Center for Food Safety and Applied Nutrition (CFSAN) and the Center

for Drug Evaluation and Research (CDER) as part of the focal research areas of excitatory amino acids/mediators of aging and neuroanatomical susceptibility. Progress to date includes the development and validation of neurochemical, neuropathological and behavioral methods for assessing alterations in amino acid neurotransmitters, dopamine release and specific neurohistological and behavioral indices associated with the N-methyl-D-asparate (NMDA)/glutamate receptor system. Studies of the developmental effects of domoic acid and the application of quantitative risk assessment procedures were published.

A cooperative research and development agreement (CRADA) between NCTR and AstraZeneca, instituted to leverage FDA/NCTR resources, continues to provide valuable information concerning the effects of long-term NMDA receptor and/or sodium channel blockade on neurobehavioral endpoints in the developing monkey. This study represents a landmark effort in neurobehavioral developmental studies and demonstrates that specific brain functions can be severely, yet selectively, affected by chronic exposure to compounds that act at the NMDA receptor complex and/or fast sodium channels. In addition, these studies extend the research database with NMDA receptor blockers into the area of potential neuroprotective agents, and further utilize NCTR's unique nonhuman primate behavioral testing capabilities. The results of these studies will provide data concerning the neurological substrates involved in the development of specific complex brain functions and suggest potential functional domains that should be of concern with other agents having similar or overlapping modes of action.

The excitatory amino acids/mediators of aging and neuroanatomical susceptibility to neurotoxicants focal area has identified the hypothalamus as an important sensitive target. The Division of Neurotoxicology is collaborating with the Division of Biochemical Toxicology at NCTR, as well as the National Institute of Environmental Health Sciences (NIEHS), through an Inter-agency Agreement (IAG), to study potential endocrine disrupter compounds. Neurotoxicology division staff have designed and implemented studies of the effects of genistein, methoxychlor, nonylphenol, and ethinyl estradiol (estrogenic compounds) and vinclozolin (an androgenic compound) on the neurohistological structure of sexually dimorphic regions of the hypothalamus. Companion studies are being conducted on male and female reproductive behaviors known to reflect the neurotoxicological alterations produced by estrogenic and androgenic hormones. These studies have identified a common pattern of alterations among the estrogenic compounds studied thus far: feminization of the male hypothalamus, with no effect on the female hypothalamus. Genistein-treated animals also exhibited mild reductions in male reproductive behaviors, while nonylphenol-treated male rat pups had significantly less circulating testosterone, which might explain the hypothalamic feminization that was observed. Vinclozolin was without effect on hypothalamic structure in males or females at the doses studied.

The neurohistochemical methods development and applications focal area has made considerable progress. Accomplishments include publishing the final evaluation and characterization of Fluoro-Jade as a marker of brain pathology. This study, in addition

to characterizing the affinity that degenerating neurons have for Fluoro-Jade, demonstrated other brain structures, including astrocytes and beta-amyloid plaques, also have an affinity for the Fluoro-Jade marker following disease or neurotoxic insult. The next generation fluorescent marker of neuronal degeneration, Fluoro-Jade B, was also characterized. This homologue of Fluoro-Jade possessed the highest specific affinity for degenerating neurons, resulting in the highest definition staining of fine degenerate neuronal processes. Another publication described the development and utility of Black-Gold, another novel histochemical tracer. This unique aurohalophosphate complex can be used for the rapid, high resolution staining of normal and pathological myelin. These unique tracers of neurotoxicity have been used in studies to resolve the respective time course of neuronal and myelin degeneration following exposure to excitotoxicants (*e.g.* kainic acid). In certain brain regions, neuronal degeneration was detected as early as four hours and myelin changes as early as two hours after kainic acid exposure. By two months post-exposure, most of the degenerated neurons and myelin had been resorbed.

Under the monoamine focal area of research, methods for assessing the neurotoxicity of the anorectic agent, d-fenfluramine, as well as the related drugs amphetamine, ephedrine, methylenedioxymethamphetamine (MDMA), and methamphetamine (METH) have been developed. Neurochemical biomarkers including concentrations and release of monoamine and excitatory amino acids, as well as neuropathological (*e.g.*, nerve terminal degeneration), behavioral (both spontaneous and operant behaviors), and body temperature have been established for the quantitative assessment of monoamine neurotoxicity. Data generated from multiple species exposed to doses of MDMA have been used to develop a biologically based, dose-response model for the quantitative risk assessment of neurotoxicants. This model, which allows the use of continuous data, was used as an example by recent review committees (*e.g.*, the National Research Council and the International Life Sciences Institute [ILSI]) to exemplify quantitation of the risk assessment process for neurotoxicants.

In conjunction with colleagues at CDER and the NIEHS, the multispecies neurotoxicological assessment of several anti-HIV agents (*e.g.*, dideoxycytidine [ddC], dideoxyinosine [ddI] and 3'-azido-3'deoxythymidine [AZT]) and the anti-tuberculosis agent, isoniazid, are nearing completion under the axonal transport/energy disruption focal research area. Neurophysiological (*i.e.,* nerve conduction studies), behavioral (both operant and spontaneous) and histological (*e.g.,* glial fibrillary acidic protein [GFAP], immunocytochemistry, degeneration-specific stains, and c-*fos* activation) methods have been applied to assess the effects of energy disruptors/transport inhibitors. Publications described the first animal model of ddI-induced peripheral neuropathy and the time course of its histological effects. A recent publication, co-authored with the National Cancer Institute (NCI) collaborators, emphasizes the potential of AZT as a transplacental carcinogen.

In cooperation with colleagues at CFSAN, the essential trace metal, manganese, is being evaluated with techniques originally developed for use with trimethyltin and methylmercury under the oxidative stress focal research area. The relationship

between organometal-induced neurotoxicity (*e.g.,* methylmercury [MMT], triethyllead, and trimethyltin) and oxidative stress has been examined with the newly developed *in vitro/in vivo* probe, dichlorofluorescein. Generation of free radicals during oxidative stress has been correlated with lipid peroxidation, superoxide dismutase (SOD), changes in neurotransmitter receptor binding and alterations in cellular activity at the molecular level (c-*fos*, heat shock proteins). These techniques were applied to other selective neurotoxicants such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and methamphetamine; and have been utilized along with neurohistological methods and behavioral assessments of memory and learning. Inhibition of neuronal nitric oxide synthase by 7-nitroindazole or by use of neuronal nitric oxide synthase knockout mice reduced their sensitivity to METH-induced neurotoxicity. Reports have been published on the oxidative-stress-producing potential of manganese and the importance of valence on the neurotoxic potency of metals. Additionally a recent publication described increased antioxidant enzyme activities following exposure to the food contaminant 3-NPA.

It is vitally important to develop appropriate animal models for use in interspecies comparisons of the effects of neuroactive agents and this need led previously to the development of automated systems for administering complex behavioral tasks to laboratory animals as well as humans. These kinds of instruments will prove very useful in aspects of post-market surveillance for newly approved drugs (in the periodic assessments of treatment efficacy and toxicity), and the identification of behavioral traits that are specific for a given clinical entity, *i.e.*, attention deficit/hyperactivity disorder (ADHD), Downs syndrome, etc. These tasks are usually identical or very similar across species. The maintenance of task continuity across species allows for the quantitative determination of similarities and differences in complex brain function and assists in the extrapolation of data from laboratory animals to humans. Performance of several of these tasks correlates significantly with IQ in humans, and identifies ADHD in children. These observations serve to validate their use in studying important aspects of brain function in animals. The NCTR's Complex Brain Function Laboratory at the Arkansas Children's Hospital continues to further define normative and clinical (*i.e*., ADHD) data for children performing NCTR's Operant Test Battery. In addition to the development and validation of the biomarkers of effect, the modulation of neurotoxicological outcome by age (development and senescence), nutritional status and body temperature have been examined. Previous research described hyperactivity resulting from developmental cerebellar stunting that was particularly prominent in males. The compounds investigated resulted in a 5-10% decrease in cerebellar weight and included: retinoic acid, dexamethasone, and methylazoxymethanol. These effects are being evaluated further as a potential animal model of ADHD. Future research includes investigating further exposure to these compounds with regard to progression of hyperactivity across development, assessment of social behavior, impulsivity, and attention.

The neurotoxicology research staff have enhanced scientific exchange by serving on several interagency committees as FDA/NCTR representatives. These committees include the Interagency Committee on Neurotoxicology (co-chair responsibilities), the

FDA Intercenter Neurotoxicity Working Group, ILSI Working Group on Human Variability, FDA's Senior Science Council, the Committee for the Advancement of FDA Science (CAFDAS), "Red Book II" FY98 revision, the World Health Organization (WHO) International Agency for Research on Cancer (11 monographs on therapeutic drugs), and Society of Toxicology's Workshop on Harmonization of Cancer and Non-Cancer Risk Assessment. In addition, members of the staff have co-organized several national or international conferences including symposia (ADHD: Characteristics, Interventions and Models), and the "Fourth International Conference on Neuroprotective Agents" and "Cellular and Molecular Mechanisms of Drugs of Abuse" which resulted in published, peer-reviewed proceedings. These outreach conferences brought together stakeholders from government, industry and academia for information exchange and consensus building concerning methods development and risk assessment procedures for neurotoxicants.

FY2000 GOALS

The overall goals of the Division of Neurotoxicology are to develop and validate quantitative biomarkers and immediate precursor events of neurotoxicity and to utilize these to elucidate toxic modes of action. This will increase the certainty of assumptions underlying human risk assessments for neurotoxicants. The strategy for achieving these goals has been to develop a multidisciplinary approach integrating neurochemical/neurobiological, neuropathological, neurophysiological, and behavioral assessments to determine effects and modes of neurotoxicity. Unique features of the neurotoxicology research efforts at NCTR include the capability to determine target tissue concentrations and cellular interactions of neurotoxicants, and to reduce the uncertainty of extrapolating data across species by effectively using rodent and nonhuman primate animal models as well as humans whenever possible.

Over the last decade, expertise, equipment and facilities have been woven together to pursue the overall goals of neurotoxicology research through six primary objectives or focal research areas. These focal areas have been developed based on prevailing scientific understanding and on the importance of each area to regulatory concerns: 1) excitatory amino acids as mediators of development, aging, and neuroanatomical susceptibility to neurotoxicants; 2) the role of aromatic monoamines in neurotoxicity; 3) disruptors of energy metabolism and axonal transport; 4) oxidative-stress-induced neurotoxicity; 5) interspecies extrapolation and validation of animal models; and 6) development, validation, and application of novel neurohistochemical tracers. These focal areas include mechanistically based approaches for defining and understanding the potential for a broad range of drugs and other chemicals to produce neurotoxic effects. In some instances, the interaction of chemicals and age (development or senescence) has been investigated and this knowledge has been used to better understand developmental neurotoxicity and as an approach to elucidate the pathogenesis of neurotoxicants.

FY2000 PLANS

Several research projects in the various focal research areas are scheduled for initiation in FY2000. Within the excitatory amino acid/mediators of neuroanatomical susceptibility to neurotoxicants area, the Endocrine Disrupters studies will continue. The promising data relating neonatal hormonal conditions to changes in adult reproductive behaviors and alterations of hypothalamic structure will be completed for genistein, methoxychlor, nonylphenol, ethinyl estradiol, and vinclozolin. Studies of the biochemical events occurring during the apoptotic and proliferative sexual differentiation stages of the developing hypothalamus also will be initiated.

The neurohistochemical methods development and applications focal area plans a number of follow-up as well as new studies. Although the affinity of Fluoro-Jade B for degenerating neurons has been fully characterized, its affinity for non-neuronal structures (*e.g.,* astrocytes, amyloid plaques, meninges, microglia, hepatocytes) following disease or toxicant exposure will require additional characterization. The chemical isolation and identification of Fluoro-Jade B is a logical follow-up study. A new neurohistochemical tracer, which will be developed and characterized, is Fluor-Opal, a fluorescent stilbene salt. This tracer allows chemical stimulation of selective brain regions, followed by observation of behavioral consequences and then histological confirmation of both the brain region involved and its connectivity to other brain regions. Documentation of EEG changes induced by Fluor-Opal injection into different brain regions may be desirable. As part of the Histochemical Test Battery, the recently developed markers of brain pathology will be applied to resolve the neuropathological potential of two other classes of putative neurotoxicants. One class is the inhibitors of oxidative respiration and the other class is comprised of acetylcholinesterase inhibitors. Collaborations are also planned with investigators at the University of Arkansas for Medical Sciences (UAMS), including developing a model of neonatal hypoxia with application to evaluate potential neuroprotective agents, resolving whether abnormal sensory stimulation induces neuronal apoptosis in the neonatal rat brain; and application of the aforementioned markers of brain pathology to human brain autopsy tissue to compare the pathologies resulting from disease processes with those associated with neurotoxic insults.

In the aromatic monoamine focal area, the influence of body temperature on d-fenfluramine- and norfenfluramine-induced neurotoxicity will be explored and studied along with other stimulants (*e.g.*, methylphenidate and ephedrine) in collaboration with CDER. In addition, it recently has been observed that excitatory amino acids and aromatic monoamines released by substituted amphetamine administration induce seizure activity that results in neurodegeneration of non-dopaminergic neurons in the cortex, thalamus, and limbic system. Reverse transcripase-polymerase chain reaction (RT-PCR) and DNA arrays methods are being employed to identify potential neurotransmitter synthesis and receptor proteins, structural proteins, and neurotrophic factors associated with the substituted amphetamine-induced acute and subsequent long-term alterations in forebrain. In collaboration with CDER and pharmaceutical sponsors, the development of a monkey model of d-fenfluramine-induced cardiotoxicity

(valvular changes) is nearing completion. In addition, studies on the seizure-genic effects and neurotoxicity of amphetamine and other substituted amphetamines have been initiated.

For the energy disruption focal area, data demonstrating the utility of animal models for the study of anti-HIV therapeutics (*e.g.*, ddI, ddC, and d4t) will be published (in collaboration with NIEHS and CDER). A manuscript that examines the fetal disposition of dideoxy-didehydrothymidine (d4T) and evaluates the monkey as a model to study the peripheral neuropathy-producing effects of thalidomide and ddC will be published in collaboration with NIEHS and CDER. Data indicating that AZT is incorporated into fetal tissue after maternal administration has been published and further studies in conjunction with personnel from the Division of Biochemical Toxicology (NCTR) will address this important issue of transplacental carcinogenicity with alternate techniques and additional nucleotides.

In the oxidative stress focal area, studies of the effects of manganese on the nervous system in both the adult and developing rat will be completed and published (in collaboration with CFSAN). A recently approved protocol focuses on the neurotoxic potential of Ibogaine. In collaboration with CFSAN, the neurotoxicity of three forms of iron (ferrous sulfate, carbonyl iron and NaFe-EDTA) in rodents will be evaluated to provide guidance as to which form would produce the lowest risk of toxicity as a food supplement. In collaboration with CFSAN, 3-nitropropionic acid (3-NPA), a foodborne agent known to produce mitochondrial dysfunction, will be used to develop a chemically induced rat model of ischemic-hypoxia. In order to validate the rat model of ischemic-hypoxia, further studies will be undertaken on the 3-NPA-induced neurotoxicity and the neuroprotective role of L-carnitine. In the interspecies extrapolation and validation of animal models focal area, validation studies on the acute effects of representative drugs in the NCTR Operant Test Battery will continue in the monkey and rat as will studies on the chronic effects of prototypic drugs *(e.g.*, methylphenidate) used in the treatment of ADHD, and agents used to treat childhood epilepsy (*i.e.,* remacemide, phenytoin).

Development of neurotoxicological knowledge bases and biologically based dose response (BBDR) models is an integral component of the overall scheme to derive predictive values for human risk. Knowledge bases are accumulations of data that have predictive values that reliably extend beyond individual data elements within a database. Predictive capabilities are achieved through the application of artificial intelligence programs such as neural networks, machine learning, expert systems, or other approaches currently being used and developed. The foundation of knowledge bases consists of biological endpoints (*e.g.*, neuropathological, neurophysiological, neurochemical, molecular biological, and behavioral), data relative to modes of action, structure-activity relationships (SAR), target-tissue concentrations, and physical/chemical properties of the agent. Hence, the prediction of human risk can be derived from the working model by assembling information in an ascending order of complexity from method-, agent-, or concept-driven research to strategies for prediction (*e.g.*, SAR and species extrapolation models) to databases. A complete database can be envisioned as the product of interactive and iterative processes between the several

foundation components (*e.g.*, endpoints and modes of action). In the process of developing knowledge bases from various data sources via quantitative risk assessment procedures, deficits in existing data will be identified that will determine directions for new research priorities. Subsequent studies can then be conducted to fill these identified data gaps to help complete the knowledge base.

PUBLIC HEALTH SIGNIFICANCE

The importance of the interdisciplinary, mechanistically based approach of neurotoxicology research is that it encourages the development of in-depth, integrated knowledge bases and techniques that are useful in addressing problems associated with current (*e.g.,* iron, fumonisin [FB_1], domoic acid, AZT, methylphenidate, fenfluramine, ibogaine, and ephedrine) and future agents of regulatory concern.

As stated in the Office of Technology Assessment (OTA) document on neurotoxicity (April 1990) and two subsequent NCTR Science Advisory Boards, NCTR has the facilities, equipment, and personnel to expand interdisciplinary research in neurotoxicity and to conduct research related to therapeutic drugs and food additives. Although neurotoxicology research at NCTR currently represents a major portion of FDA's neurotoxicology efforts, it must maintain its flexibility in order to deal effectively with future FDA needs. The following four-fold plan has been developed to allow neurotoxicology research to keep pace with FDA's responsibility to assure safe and effective drugs, foods, devices, and cosmetics. First, the Division must continue and enhance interactions with other FDA centers in order to better understand and address FDA regulatory concerns (*e.g.*, the FDA Intercenter Neurotoxicity Working Group). Second, the Division needs to expand its efforts in interdisciplinary and fundamental research approaches, especially in the molecular and interspecies areas, in order to validate appropriate animal models and quantitative risk assessment techniques for neurotoxicants. Third, it will continue to develop and validate improved quantitative risk assessment procedures with broad applicability, and fourth, the Division needs to continue to develop predictive system and knowledge base approaches to solve neurotoxicological problems. Integration of neurotoxicology research, FDA-wide, will provide the scientific basis necessary for sound regulatory decisions.

ACTIVE PROJECTS FY1999

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0212213	**ADDEND: A Pilot Study to Assess the Effect of Developmental Genistein Exposure on Sexually Dimorphic Behaviors** To determine whether pre/neonatal exposure to genistein, a compound with estrogenic properties, will alter imprinting of sex differences in behavior.	Ferguson, Sherry A.*
E0212215	**ADDEND: Neurotoxicological Effects of Exposure to Estrogenic Compounds during Development: II. Genistein** 1. To determine whether developmental exposure to genistein may modify the sexually dimorphic areas of the adult rodent brain; 2. To compare neurochemical and neurohistological biomarkers of genistein exposure for their relative sensitivity and concordance.	Scallet, Andrew C.*
E0212313	**ADDEND: A Pilot Study to Identify Cost-Effective Sexually Dimorphic Behaviors Sensitive to the Effects of Development Exposure to Estrogenic Compounds (Methoxychlor)** To identify easily-automated, cost-effective behavioral assays which are sexually dimorphic and sensitive to developmental exposure to environmental estrogens.	Ferguson, Sherry A.*
E0212315	**ADDEND: Neurotoxicological Effects of Exposure to Estrogenic Compounds during Development: I. Methoxychlor** 1. To determine whether developmental exposure to methoxychlor may modify the sexually dimorphic areas of the adult rodent brain; 2. To compare neurochemical and neurohistological biomarkers of methoxychlor exposure for their relative sensitivity an concordance.	Scallet, Andrew C.*
E0212513	**ADDEND: A Pilot Study to Assess the Effect of Developmental Nonylphenol Exposure on Sexually Dimorphic Behaviors** To determine whether pre/neonatal exposure to nonylphenol, a compound with estrogenic properties, will alter sex differences in behavior.	Ferguson, Sherry A.*
E0212515	**ADDEND: Neurotoxicological Effects of Exposure to Estrogenic Compounds during Development: III. Nonylphenol** 1. To determine whether dvelopmental exposure to nonylphenol may modify the sexually dimorphic areas of the adult rodent brain; 2. To compare neurochemical and neurohistological biomarkers of nonylphenol exposure for their relative sensitivity and concordance.	Scallet, Andrew C.* Meredith, John M.
E0212613	**ADDEND: A Pilot Study to Assess the Effect of Developmental Vinclozolin Exposure on Sexually Dimorphic Behavior** To determine whether pre/neonatal exposure to vinclozolin, a compound with potential estrogenic properties, will alter sex differences in behavior.	Ferguson, Sherry A.* Weis, Constance C.
E0212615	**ADDEND: Neurotoxicological Effects of Exposure to an Anti-Androgenic Compound during Development: Vinclozolin** 1. To determine whether developmental exposure to vinclozolin may modify the sexually dimorphic areas of the adult rodent brain; 2. To compare neurochemical and neurohistological biomarkers of vinclozolin exposure for their relative sensitivity and concordance.	Scallet, Andrew C.* Meredith, John M.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0212913	**ADDEND: A Pilot Study to Assess the Effect of Developmental Ethinyl Estradiol Exposure on Sexually Dimorphic Behaviors** To determine whether pre/neonatal exposure to ethinyl estradiol, a compound with potential estrogenic properties, will alter sex differences in behavior.	Ferguson, Sherry A.* Weis, Constance C.
E0212915	**ADDEND: Neurotoxicological Effects of Exposure to Estrogenic Compounds During Development: V. Ethinyl estradiol** 1. To determine whether developmental exposure to ethnyl estradiol may modify the sexually dimorphic areas of the adult rodent brain; 2. To compare neurochemical and neurohistological biomarkers of ethinyl estradiol exposure for their relative sensitivity and concordance.	Scallet, Andrew C.* Meredith, John M.
E0213213	**ADDEND: The Effects of Developmental/Chronic Genistein Exposure over Multiple Generations on Maternal, Play, Mating/Reproductive Behaviors and Neurochemical Measures** To determine whether chronic exposure of rats over multiple generations to genistein, a compound with potential estrogenic properties, will alter maternal behavior, play behavior of either sex, the female lordosis response, male mating behavior or the amphetamine-induced release of striatal dopamine, which is known to be estrogen-modulated.	Ferguson, Sherry A.* Flynn, Katherine M. Gough, Bobby J.
E0213513	**ADDEND: The Effects of Developmental/Chronic Nonylphenol Exposure over Multiple Generations on Sexually Dimorphic Behaviors, and Neurochemical Measures** To determine whether chronic exposure of rats over multiple generations to nonylphenol, a compound with potential estrogenic and/or androgenic properties, will alter maternal behavior, the female lordosis response, male mating behavior, sodium solution intake, amphetamine-induced release of the striatal dopamine, or serum levels of testosterone and estradiol in males.	Ferguson, Sherry A.* Flynn, Katherine M. Gough, Bobby J.
E0250201	**Neurotoxicological and Behavioral Assessment of the Human Immunodeficiency Virus (HIV) Suppressors 2',3'-dideoxycytidine (ddC) and Thalidomide in Rhesus Monkeys** To assess the neurotoxicity and neurobehavioral effects of chronic treatment with the anti-HIV agents 2',3'-dideoxycytidine (ddC) and thalidomide in rhesus monkeys.	Patterson, Tucker A.* Paule, Merle G. Sandberg, Jennifer A. Schmued, Laurence C. Slikker, William Hill, Barbara Sheevers, Hillary Zielinski, Walt
E0250211	**ADDEND: Additional Pathology Requirements – Neutotoxicological and Behavioral Assessment of the HIV Suppressors 2',3'-ddC and Tahlidomide in Rhesus Monkeys** This addendum allows for more tissues to be processed following necropsy in order to garnish additional data from the study.	Patterson, Tucker A.*

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0250221	**ADDEND: T.O. #682 – Statistical Analysis of ddC/Thalidomide Behavior Tasks: Neurotoxicological and Behavioral Assessment of the HIV Suppressors ddC and Thalidomide in Rhesus Monkeys** Task order initiated for the statistical analysis of the behavioral task endpoints of monkeys exposed to ddC and Thalidomide versus a control group.	Paule, Merle G.*
E0250301	***In Vitro* Cellular Toxicity Studies of the Dideoxynucleoside Antiretrovirals, ddI, ddC, 3TC and dT** 1. To utilize an established cell model for investigation of ddN-induced peripheral neuropathy and optimization of experimental methods and to apply these optimized investigative techniques to a novel cell model, Schwann cells; 2. To examine the metabolism of various ddN's in the PC12 and, subsequently, the Schwann cell model; 3. To evaluate and compare the effects of sublethal concentrations of ddN's on cell functions in both cell models, compared to effects of AZT, a dideoxynucleoside that does not produce a peripheral neuropathy; 4. To determine the relative contribution of identified ddN-induced alterations in cellular functions to toxicity.	Schnellmann, Jennifer D.* Bowyer, John F. Slikker, William
E0280001	**Development of a Nonhuman Primate Model for Studying the Consequences of Long-term Anticonvulsant Medication on Complex Brain Functions (97032)-ASTRA CRADA** 1. To establish acquisition curves for several operant behaviors in juvenile rhesus monkeys during chronic oral exposure to two anticonvulsant agents and vehicle; 2. To determine whether such exposure results in any significant changes in the acquisition and performance of these operant and other observable behaviors; 3. To determine whether such exposure results in any significant changes in clinical chemistry or ophthalmic parameters; 4. To determine plasma distribution profiles and concentrations for each of these agents at various stages of chronic exposure.	Paule, Merle G.* Binienda, Zbigniew K. Gillam, Michael P. Hammond, Tim Pearson, Edwin Popke, Jon Slikker, William Somers, Mary D.
E0280011	**ADDEND: Development of a Nonhuman Primate Model for Studying the Consequences of Long-term Anticonvulsant Medication on Complex-Brain Functions** Determine whether the effects of chronic ramacemide treatment are due to reversible effects linked to daily drug exposure or are due to irreversible Central Nervous System (CNS) toxicity. Proposing to monitor behavioral acquisition in subjects during six months of reduced drug exposure. Requesting extension of project requiring addn'l housing/maintenance and research time.	Paule, Merle G.* Popke, Jon
E0663306	**ADDEND: Preliminary Studies for Determining the Effects of Chronic Cocaine Exposure during Pregnancy on the Behavior of Offspring in Monkeys** Increase the number of offspring in the total gestational exposure (TGE) group to ten. Requesting that 10 nonpregnant animals be maintained under chonic cocaine treatment while they are in the breeding program until at least 10 viable offspring are available. Requesting another 7 animals for inclusion in control group to bring the total to 10.	Paule, Merle G.* Binienda, Zbigniew K.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0683700	**Effects of Chronic Methylphenidate (Ritalin) Administration on 'cognitive' Functions in the Rhesus Monkey** To determine whether chronic treatment with relevant doses of the therapeutic agent methylphenidate (Ritalin) will result in detectable changes in specific 'cognitive' abilities in a nonhuman primate model of complex brain function.	Paule, Merle G.* Gillam, Michael P. Slikker, William
E0691401	**Validation of the NCTR Rodent Operant Test Battery as an Adjunct to the NCTR Primate Operant Test Battery: Implications for the Areas of Risk Assessment and Prediction of Neurobehavioral Toxicity** 1. To determine the acute effects of a variety of prototypic psychotropic agents on rodent performance in an operant test battery (OTB) containing tasks designed to model several complex brain functions; 2. To determine the relative sensitivities of the behavioral endpoints monitored in the rodent OTB to pharmacological disruption; 3. To compare and contrast the acute effects of these psychotropic agents on rodent and primate OTB performance to determine the degree to which behavioral findings in rodents can be extrapolated to primates. 4. To validate the use of rodent operant performance as useful predictors of neurobehavioral toxicity; 5. To add to existing knowledge of the neurochemical and neurophysiolgoical basis of complex brain functions.	Paule, Merle G.* Fogle, Charles M.
E0691411	**ADDEND: Validation of the NCTR Rodent Operant Test Battery as an Adjunct to the NCTR Primate Operant Test Battery: Implications for the Areas of Risk Assessment and Prediction of Neurobehavioral Toxicity** Transfer of 24 rats from P00327 to E06914.01 and add fenfluramine to the list of agents to be evaluated under E06914.01. Transfer of animals will enable researcher to evaluate the acute effects of the various psychotropic agents outlined in E06914.01.	Paule, Merle G.* Fogle, Charles M.
E0691421	**ADDEND: Validation of the NCTR Rodent Operant Test Battery as an Adjunct to the NCTR Primate Operant Test Battery: Implications for the Areas of Risk Assessment and Prediction of Neurobehavioral Toxicity** Addendum submitted to administer one additional known or putative neurotoxicant to rats and then to monitor their behavior for approximately one additional month.Requires additional dosing requirements.	Paule, Merle G.* Fogle, Charles M.
E0691431	**ADDEND: Validation of the NCTR Rodent Operant Test Battery as an Adjunct to the NCTR Primate Operant Test Battery: Implications for the Areas of Risk Assessment and Prediction of Neurobehavioral Toxicity** To allow drugs to be administered orally - change route of administration of drugs.	Popke, Jon*

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0692601	**Implementation of Molecular Biological Techniques for Assessing Changes in Neurogrowth/ Neurotrophic Factors after Exposures to Neurotoxicants and Other Substances** Select and produce/obtain cDNA and RNA probes for detecting changes in messenger RNA (mRNA) levels for the various neurogrowth/neurotrophic factors (NTFs) which are likely to be involved in either secondary mechanisms of neurotoxicity or repair after neurotoxicant insult. Detect changes in NTF mRNAs after insult to neurotoxicants and other substances, and determine if these are the same for very young and older animals.	Bowyer, John F.* Slikker, William Tank, A.W. Goldman, Neil
E0693001	**Estimating Quantitative Neurotoxicity Risk from Domoic Acid Exposure** To correlate pharmacokinetic profiles of single and multiple doses of domoic acid with associated quantitative neurohistological and behavioral effects in non-human primates; 1) To identify genetic factors modulating domoic acid sensitivity in Wistar rats; 2) To identify neurochemical biomarkers of domoic acid exposure and damage.	Scallet, Andrew C.* Ali, Syed F. Hall, Sherwood Johannessen, Jan Paule, Merle G. Rountree, Robert L. Schmued, Laurence C. Slikker, William Sobotka, Thomas
E0697901	**Use of the NCTR Nonhuman Primate Operant Test Battery (OTB) as a Predictor of Acute Neurobehavioral Toxicity: Pharmacological Manipulation at Specific Neurotransmitter Receptor Subtypes** 1. To further explore the extent to which the use of operant behavioral techniques in nonhuman primates can serve to reliably model the effects of compounds selected to act on specific neurotransmitter systems; 2. To determine the acute dose-effect relationships of several drugs believed to act primarily at subtypes of specific neurotransmitter receptors using rhesus monkey OTB performance; 3. To characterize the relative sensitivities of the various behavioral endpoints in the NCTR OTB to pharmacological manipulation of specific neurotransmitter systems and to add new tasks to the NCTR OTB; 4. To more thoroughly characterize the role of specific neurotransmitter systems in the expression of complex brain functions through the pharmacological manipulation of specific receptor subtypes of some of the known major neurotransmitter systems; 5. To determine if the acute behavioral effects of the exogenous compounds of interest differ with regard to gender in the rhesus monkey.	Paule, Merle G.* Gillam, Michael P.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0698301	**Effects of Ibogaine on Neurotransmitter Systems, Generation of Free Radicals and Nitric Oxide Synthase Activity: Correlation with Neurohistological Evaluations in Mouse and Rat Brain** 1. To determine the effects of ibogaine on dopamine, serotonin and their metabolite concentrations in different regions of mouse and rat brain; 2. To determine the effects of ibogaine on reactive oxygen species (ROS) and lipid peroxidation in different regions of mouse and rat brain. 3. To determine the effects of ibogaine on the activities of several antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione levels in different regions of mouse and rat brain. 4. To evaluate the effects of ibogaine on the activity of nitric oxide synthase (NOS) in different regions of mouse and rat brain. 5. To determine the levels of ibogaine, noribogaine and neurohormone, prolactin and corticosterone in plasma of mouse and rat. 6. To evaluate the neurohistorical effects of ibogaine in different brain regions in the mouse and the rat and to correlate them with any neurochemical alterations.	Ali, Syed F.* Duhart, Helen M. Hussain, Saber M. Klein, Michael Lipe, George W. Mukherjee, Asoke Newport, Glenn D. Rountree, Robert L. Scallet, Andrew C. Schmued, Laurence C. Slikker, William Ye, Xuemin
E0698311	**ADDEND: The Effects of Ibogaine on Neurotransmitter Systems, Generation of Free Radicals and Nitric Oxide Synthase Activity: Correlation w/Neurohistological Evaluations in Mouse and Rat Brains** Investigate if direct infusion of compounds into the brain produces similar changes in the neurotransmitter system in rats - Will inject ibogaine, noribogaine and the structurally related compound harmaline directly into the brain and will evaluate the changes in neurotransmitter levels. Requesting additional 24 male adult Sprague Dawley rats.	Ali, Syed F.*
E0698321	**ADDEND: The Effects of Ibogaine on Neurotransmitter Sytems: Correlation with Body Temperature and Electroencephalogram (EEG)** To investigate what effect ibogaine might have on the electroencephalogram profile along with the time course of temperature changes in rats exposed to this compound. Would like to inject ibogaine 50 mg/kg, i.p. in five male adult Sprague-Dawley rats instrumented for the EEG and temperature recording as described in the protocol P00404. Addn'l resources will be labor for Dr. Binienda as a CO-PI - no other addn'l resources required.	Ali, Syed F.* Binienda, Zbigniew K.
E0699201	**Validation Study of the Physiologically-based Pharmacokinetic (PBPK) Model for Description of Low-dose, Long-term Exposure of 2,4-Dichlorophenoxyacetic Acid (2,4-D) Dosimetry in the Central Nervous System (CNS)** To obtain CNS pharmacokinetic profiles of 2,4-D transport in the rat after low-dose, chronic dosing (28 days). The data will be used to validate the previously developed PBPK model which simulates the uptake, distribution, and clearance of 2,4-D.	Slikker, William* Binienda, Zbigniew K. Duhart, Helen M. Kim, Chung Lipe, George W.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0701001	**Metabolic Correlates of the Neurotoxicity Associated with Exposure to the Mitochondrial Inhibitor 3-nitropropionic Acid (3-NPA) in the Rat: The Role of Free Fatty Acids (FFA)** 1. To evaluate the acute effects of the mitochondrial inhibitor 3-NPA on brain metabolic activity using elecrophysiological, neurochemical, and neurohistological endpoints:a) spontaneous electrical brain activity and averaged visual evoked potentials; b) FFA concentration in different brain regions; c) brain regional monoamine neurotransmitter concentrations: dopamine, serotonin, and their metabolites; d) microscopically detectible neuronal damage; 2. To assess the possible neuroprotective effect of L-carnitine in the rat model of 3-NPA-induced histotoxic hypoxia.	Binienda, Zbigniew K.* Ali, Syed F. Kim, Chung Nickols, Jess Rountree, Robert L. Scallet, Andrew C. Slikker, William
E0701301	**Development and Validation of a Neurohistochemical Test Battery for Resolving the Distribution of Lesions and the Underlying Mechanisms of Action of Neurotoxicants** 1. To develop and validate a battery of conventional and novel histochemical techniques for resolving the nature, distribution and underlying mechanisms of brain damage resulting from exposure to FDA relevant neurotoxicants; 2. To localize throughout the central nervous system histochemical and pathological changes resulting from exposure to different classes of neurotoxicants, and 3. By correlating a compound's putative mode of action with a characteristic histochemical profile, develop the ability to predict the neuroanatomical regions at risk and the potential functional consequences of the neurotoxicant of interest.	Schmued, Laurence C.* Ali, Syed F. Bowyer, John F. Hopkins, Keri J. Scallet, Andrew C. Slikker, William Hall, Sherwood
E0701901	**Experimental Autoimmune Prostatitis: Implications for the prevention and treatment of Inflammatory and Neoplastic Disorders of the Prostate Gland** 1. To induce an experimental autoimmune prostatitis in male rhesus monkeys by immunizing animals with homogenates of monkey prostate gland and mixed with Freund's adjuvant; 2. To identify the target proteins of the induced autoimmune prostatitis by using the immune sera (IgG) from the above animals to i) screen prostate homogenates by Western immunoblot analyses and ii) screen a monkey prostate cDNA expression library.	Binienda, Zbigniew K.* Chatta, Gurkamal S. Hardin, James
E0701911	**ADDEND: Experimental Autoimmune Prostatitus: Implications for the Prevention and Treatment of Inflammatory and Neoplastic Disorders of the Prostate Gland** Requesting to immunize three more primates with purified proteins and simultaneously compare them with animals immunized with the whole organ homogenates. Animals will be furnished through UAMS via monies from an NIA pilot grant.	Binienda, Zbigniew K.* Chatta, Gurkamal S.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0702401	**Evaluation of the Neurotoxic Effects and Determination of the Mechanisms of Induction of Limbic Seizures Produced by Amphetamine and Related Compounds** 1. To measure the effects of dose and age on the susceptibility of amphetamine-induced limbic-type seizures in three different strains of rat and mouse, and identify areas in the brain, in particular the limbic system, where cell death and neuroplastic changes occur after amphetamine-induced seizures; 2. Determine the seizure genic capabilities of amphetamine, phentermine, methylphenidate and ephedrine in rat and mouse, the extracellular brain levels of these compounds necessary to induce seizures, and whether hyperthermia plays a role in the seizure induction; 3. Determine via brain microdialysis if extracellular glutamate levels are elevated in the limbic system (hippocampal rudiments and piriform cortex) prior to and during seizures induced by amphetamines; 4. Elucidate the role the noradrenergic, as well as the glutamatergic, system plays in seizures generated by amphetamines. Furthermore, begin to determine how agonists and antagonists of these two neurotransmitter systems can potentiate the seizure genesis of amphetamines.	Bowyer, John F.* Binienda, Zbigniew K. Davies, David L. Ferguson, Sherry A. Newport, Glenn D. Peterson, Steven L. Schmued, Laurence C. Slikker, William
E0702601	**Preliminary Studies for the Effects of Chronic Dexfenfluramine Administration in the Rhesus Monkey** 1. To determine if the rhesus monkey demonstrates cardiac valve changes due to chronically administered dexfenfluramine; 2. To determine if the rhesus monkey demonstrates neurobiological changes due to chronically administered dexfenfluramine.	Slikker, William* Binienda, Zbigniew K. Bowyer, John F. Degeorge, Joseph Paule, Merle G. Schnellmann, Jennifer D.
E0702611	**ADDENDUM: Preliminary Studies for the Effects of Chronic Dexfenfluramine Administration in the Rhesus Monkey** Requesting maintenance and dosing of primates be extended for additional month.	Slikker, William*
E0703101	**Decision Making in Children with Attention Deficit Disorder** 1. To determine if children diagnosed with Attention Deficit Hyperactivity Disorder in attentive subtype, ADHD hyperactive/impulsive subtype, and ADHD combined subtype differ from each other and children with out any psychiatiric problems in their ability to delay gratification; 2. To determine if children diagnosed with ADHD inattentive subtype, ADHD hyperactive/impulsive subtype, and ADHD combined subtype, and controls differ in the degree that they discount delayed rewards using a delay of gratification procedure in which choices are made for hypothetical amounts of money; 3. To determine for each ADHD subtype, the relationshp between severity of ADHD symptoms and delay of gratification in both of the tasks mentioned above; 4. To obtain preliminary data for determining relationships between measures of delay of gratification and other commonly used measures of assessing impulsivity in children with ADHD.	Chelonis, John J.* Blake, Donna Paule, Merle G. Schulze, G E.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0704001	**Validity of Developmental Cerebellar Stunting in the Rat as a Model for Attention Deficity Hyperactivity Disorder: Behavior and Neurochemistry** 1. To identify treatments, which cause developmental cerebellar stunting, specifically those which decrease the granule cell population with few effects on Purkinje cells; 2. To confirm the increase in locomotor activity caused by developmental cerebellar stunting and to determine the degree to which this hyperactivity resembles human ADHD; 3. To identify other behavioral alterations associated with developmental cerebellar stunting and to determine the degree to which these resemble those associated with human ADHD; 4. To identify the neurochemical alterations in different brain regions resulting from the developmental insult; 5. To compare these neurobehavioral and neurochemical alterations to those exhibited by the most common rodent model of ADHD: the Spontaneously Hypertensive Rat (SHR).	Ferguson, Sherry A.* Ali, Syed F. Gough, Bobby J. Paule, Merle G.
E0704401	**Complex Brain Function in Autistic Children** To compare brain functioning among autistic children and normal functioning children using tests that assesses motivation, color/position discrimination and memory. Additionally, these measures will be compared among autistic children with various degrees of symptom severity.	Chelonis, John J.* Paule, Merle G.
P00386	**Arkansas Children's Hospital Statistical Support** Project will involve an empirical investigation of OTB performance by normal children and children identified as expressing specific clinical diagnoses including Attention Deficit Disorder with or without Hyperactivity.	Paule, Merle G.*
P00400	**Training one Science Internship Program Student in use of the Neurobehavioral Teratology Laboratory Behavioral Assessments and Standard Rodent Experimental Techniques** This project will train one new undergraduate student on the use and conduct of most of the rodent assessments contained in the Neurobehavioral Teratology Lab as well as the standard techniques used in the laboratory of sexing, culling, and tattooing litters at parturition.	Ferguson, Sherry A.*

PROJECTS COMPLETED FY1999

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0688701	**Evaluation of Constitutive and Stress-Induced Levels of Expression of Heat-Shock Proteins (HSP) in Cu/Zn-Superoxide Dismutase Transgenic Mice** 1. Determine whether there are significant differences in constitutive HSP expression in Cu/Zn-Superoxide Dismutase-transgenic mice versus non-transgenic littermate controls, C57BL/6N controls as well as CD1 controls. 2. Determine whether there are significant differences in the expression of inducible forms of HSPs after exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in superoxide dismutase (SOD)-transgenic mice versus non-transgenic littermate controls, C57BL/6N controls as well as CD1 controls. 3. Determine whether there are significant differences in the timeframe of the HSP response in SOD-transgenic mice versus non-transgenic litter-mate controls, C57BL/6N controls as well as CD1 controls. 4. Determine whether there exists differential expression of isoforms of HSP in SOD-transgenic mice versus non-transgenic mice littermate controls, C57BL/6N controls as well as CD1 contrls. 5. Evaluate if induction of HSP correlates with the depletion of dopamine in SOD-transgenic mice versus non-transgenic controls, C57BL/6N controls as well as CD 1 controls.	Ali, Syed F. * Cadet, Jean Freyaldenhoven, Timothy E. Newport, Glenn D. Slikker, William
E0688711	**ADDEND: Evaluation of Constitutive and Stress-Induced Levels of Expression of Heat Shock Proteins in Cu/Zn-Superoxide Dismutase Transgenic Mice** Requesting to use dams from E06933.01. We hypothesize that these p53 knockout mice will be resistant to MPTP and methamphetamine. We would like to determine if these mice are also resistant to MPTP and methamphetamine-induced neurotoxicity. Adult female p53 knockout mice will be injected at different doses with MPTP or methamphetamine and will be sacrificed at different times. We will use these mice as they become available under the protocol E06933.01. Since these transgenic mice were derived from C57 mice, we also will use some C57 mice as controls.	Ali, Syed F. *
E0692301	**3-Nitropropionic Acid (3-NPA) Hypoxia in the Rat: Neurochemical and Neurohistological Studies** 1. To evaluate the effects of the developmental neurotoxin 3-NPA on N-methyl-D-asparate (NMDA), dopaminergic and serotonergic systems using neurochemical methods. 2. To evaluate the neurohistological effects of calcium-mediated vs. serum–mediated stimuli on the expression of stress proteins (c-fos). 3. To correlate 3-NPA toxicity with age.	Binienda, Zbigniew K. * Ali, Syed F. Flynn, Thomas Kim, Chung Rountree, Robert L. Scallet, Andrew C.
E0692311	**ADDEND: 3-nitropropionic Acid (3-NPA) Hypoxia in the Rat: Neurochemical and Neurohistological Studies** Add electroencephalography to neurochemical and neurohistological endpoints that will be analyzed under the master project E06923.01. Requesting 5 addn'l animals (retired breeders) for methods development. Addn'l PI time estimated also.	Binienda, Zbigniew K. * Ali, Syed F. Flynn, Thomas Kim, Chung Rountree, Robert L. Scallet, Andrew C. Slikker, William

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0692321	**ADDEND: 3-nitropropionic Acid (3-NPA) Hypoxia in the Rat: Neurochemical and Neurohistological Studies** Alter the method of animal's restraint during the EEG recording. Will be using general isoflurane anesthesia to facilitate EEG collection. Alteration of a restraint method during an experimental procedure; no additional resources and time is required.	Binienda, Zbigniew K. * Flynn, Thomas Kim, Chung
E0692331	**ADDEND: 3-NPA Hypoxia in the Rat: Neurochemical and Neurohistological Studies** Proposing to apply another method, i.e., plastic embedding and sectioning and electron microscopy method, in the study. It is very important to add the plastic embedding and sectioning method to the original protocol. The work proposed in this addendum will help to perform further FDA relevant neurotoxicological studies in the future. Adding research time for Dr. Wang and pathology hours.	Binienda, Zbigniew K. * Wang, Guang J.
E0694301	**Behavioral and Neurochemical Effects of Short Course, High Dose Exposure to Methylenedioxymethamphetamine (MDMA) or dexfenfluramine (FEN) in Rhesus Monkeys** To establish acute dose-response curves for MDMA and d-FEN using performance of two groups of rhesus monkeys in the NCTR primate operant test battery (OTB). To produce long-term damage to the serotonin (5-HT) system of the forementioned monkeys via short course, high dose administration of MDMA or d-FEN.To determine whether rhesus monkeys exposed to short course, high dose MDMA or d-FEN exhibit persistent changes in CNS functioning, as quantified by changes in OTB performance.To determine if short course, high dose exposure to MDMA or d-FEN produces long-lasting changes in the acute effects of each drug (and hence long-term changes in CNS function) by establishing a second acute dose-response curve for each drug after such exposure.To demontrate possible long-term changes in both neurochemical and behavioral endpoints resulting from MDMA and d-FEN exposure in rhesus monkeys that may assist in the determination of the status of these drugs as therapeutic agents.	Paule, Merle G. * Ali, Syed F. Binienda, Zbigniew K. Gillam, Michael P. Slikker, William Grieshaber, Charles
E0694311	**ADDEND: Behavioral and Neurochemical Effects of Short Course, High Dose Exposure to MDMA or d-FEN in Rhesus Monkeys** To replace one monkey due to death and add two additional subjects to the study. No additional contract support will be required; only change is number of monkeys in each treatment group.	Paule, Merle G. *
E0694321	**ADDEND: Behavioral and Neurochemical Effects of Short Course, High Dose Exposure to MDMA or d-FEN in Rhesus Monkeys** To obtain blood samples during the short, course, high dose MDMA and d-Fen administration for the purpose of performing subsequent pharmacokinetic assays of plasma samples. Dr. Peter Clausing has also been included as a CO-PI.	Paule, Merle G. * Clausing, Peter P.

FY1999 PUBLICATIONS*

1. Ali, S.F. and Itzhak, Y. Effects of 7-nitroindazole, and NOS inhibitor on methamphetamine-induced dopaminergic and serotonergic neurotoxicity in mice. Annals of the New York Academy of Sciences, 844:122-130. Accepted: 10/1/98. **(E0698301)**

2. Binienda, Z.K., Johnson, J.R., Tyler-hashemi, A.A., Rountree, R.L., Sapienza, P.P., Ali, S.F. and Kim, C. Protective effect of L-Carnitine in the neurotoxicity induced by the mitochondrial inhibitor 3-nitropropionic acid (3-NPA). Annals of the New York Academy of Sciences, Accepted: 6/1/99. **(E0701001)**

3. Bowyer, J.F. and Davies, D.L. Changes in mRNA levels for heat-shock/stress proteins (Hsp) and a secretory vesicle associated cysteine-string (Csp 1) after amphetamine (AMPH) exposure. Annals of the New York Academy of Sciences, Accepted: 7/1/99. **(E0690301)**

4. Bowyer, J.F. and Peterson, S.L. Neuronal degeneration in the forebrain produced by amphetamine methamphetamine and fenfluramine. In Cellular and Molecular Mechanisms of Toxin Actions, Vol. 4 Site-Selective Neurotoxicity, Accepted: 7/2/99. **(E0702401)**

5. Clausing, P.P. and Bowyer, J.F. Time course of brain temperature and striatal microdialysate levels of amphetamine and dopamine in rats after multiple doses of d-amphetamine. Annals of the New York Academy of Sciences, Accepted: 6/1/99. **(E0692601)**

6. Desaiah, D., Pande, M., Vig, P.J., Cameron, J.A. and Ali, S.F. Sensitivity of brain nitric oxide synthase activity to phencyclidine, International Journal of Toxicology, Accepted: 8/1/99. **(E0698301)**

7. Eisch, A.J., Schmued, L.C. and Marshall, J.F. Characterizing cortical neuron injury with fluoro-jade labeling after a neurotoxic regimen of methamphetamine, Synapse, 30:329-333, 1999. Accepted: 11/1/98. **(E0701301)**

8. Flynn, K.M., Schreibman, M.P., Yablonsky-Alter, E. and Banerjee, S.P. Sexually dimorphic development and binding characteristics of NMDA receptors in the brain of the platyfish. General and Comparative Endocrinology, Accepted: 3/10/99. **(NA)**

9. Genter, M.B. and Ali, S.F. Age-related susceptibility to 3,3'-iminodipropionitrile-induced olfactory mucosal damage, Neurobiology of Aging, Accepted: 6/1/99. **(E0698301)**

10. Hashemi, R.R., Danley, J.M., Tyler, A.A., Slikker, W. and Paule, M.G. The quality of information granulation: Kohonen self-organizing map vs. neighborhood systems. Proceedings of the 4th Joint Conference on Information Science, Accepted: 10/5/98. **(NA)**

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* **(_ _ _ _ _ _ _ _)** ***Indicates the related NCTR project number; NA-Not Applicable***

11. Hashemi, R.R., Slikker, W. and Paule, M.G. Profiling through kohonen self-organizing map: The effect of birth weight on the performance measures of an operant test battery, ANNIE'99, Accepted: 7/7/99. **(E0703301)**

12. Holson, R.R., Adams, J. and Ferguson, S.A. Gestational stage-specific effects of retinoic acid exposure in the rat, Neurotoxicology and Teratology, Accepted: 2/15/98. **(NA)**

13. Imam, S.Z., Crow, J.P., Islam, F., Slikker, W. and Ali, S.F. Methamphetamine generates peroxynitrite and produce dopaminergic neurotoxicity in mice: protective effects of peroxynitrite scavenger FeTMPyP. Brain Research, 837(1-2):15-21, 1999. Accepted: 5/18/99. **(E0698301)**

14. Imam, S.Z., Newport, G.D., Islam, F., Slikker, W. and Ali, S.F. Selenium, an antioxidant, protects against methamphetamine-induced dopaminergic neurotoxicity. Brain Research, 818:575-578, 1999. Accepted: 12/1/98. **(E0698301)**

15. Itzhak, Y. and Ali, S.F. Effect of ibogaine on the various sites of the NMDA receptor complex and sigma binding sites in rat brain. Annals of the New York Academy of Sciences, 844:245-251, 1999. Accepted: 10/1/98. **(E0698301)**

16. Kwon, O., Newport, G.D. and Slikker, W. Quantitative analysis of free sphingoid bases in the brain and spinal cord tissues by high-performance liquid chromatography with a fluorescence detection, Journal of Chromatography B, 720:9-14, 1999. Accepted: 10/22/98. **(E0688401)**

17. Lester, D.S., Lyon, R.C., Mcgregor, G.N., Engelhart, R.T., Schmued, L.C., Johnson, G.A. and Johannessen, J. 3-Dimensional visualization of lesions in rat brain using magnetic resonance imaging microscopy. NeuroReport, 10:737-741, 1999. Accepted: 3/1/99. **(E0701301)**

18. Mayorga, A.J., Fogle, C.M. and Paule, M.G. Adaptation of a primate operant test battery to the rat: effects of chlorpromazine. Neurotoxicology and Teratology, Accepted: 6/18/99. **(E0691401)**

19. Mills, K.J., Arsah, T.A., Ali, S.F. and Shockley, D.C. Calcium channel antagonist isradipine attenuates cocaine-induced motor activity in rats: correlation with brain monoamine levels. Annals of the New York Academy of Sciences, 844:201-207, 1999. Accepted: 10/1/98. **(Ofc. of Dir./Imm. Ofc.) (E0698301)**

20. Nony, P.A., Scallet, A.C., Rountree, R.L., Ye, X. and Binienda, Z.K. 3-nitropropionic acid (3-NPA) produces hypothermia and inhibits histochemical labeling of succinate dehydrogenase (SDH) in rat brain. Metabolic Brain Disease, Accepted: 5/13/99. **(E0692301)**

21. Onaivi, E.S., Ali, S.F. and Chakrabarti, A. *In vivo* ibogaine blockade and *in vitro* PKC action of cocaine. Annals of the New York Academy of Sciences, 844:227-244, 1999. Accepted: 10/1/98. **(E0698301)**

22. Pande, M., Cameron, J.A., Vig, P.J., Ali, S.F. and Desaiah, D. Inhibition of calcium ATPase by phencyclidine in rat brain. Journal Molecular and Cellular Biochemistry, Accepted: 8/15/99. **(E0608301)**

23. Patterson, T.A., Schmued, L.C., Sandberg, J.A. and Slikker, W. Temporal development of 2',3'-dideoxyinosine-(DDI) induced peripheral myelinopathy. Neurotoxicology and Teratology, Accepted: 8/29/99. **(E02500.01)**

24. Paule, M.G. Validation of a behavioral test battery for monkeys. In: Methods and Behavioral Analysis in Neuroscience. Jerry Buccafusco, Editor, Accepted: 7/26/99. **(E0697901)**

25. Poirier, M., Patterson, T.A., Slikker, W. and Olivero, O. Incorporation of 3'--azido-3'deoxythymidine (AZT) into fetal DNA, and fetal tissue distribution of drug, after infusion of pregnant late-term rhesus macaques with a human-equivalent AZT dose. Journal of Acquired Deficiency Syndromes and Human Retrovirology, Accepted: 8/20/99. **(E0250101)**

26. Popke, J., Fogle, C.M., Allen, S.R. and Paule, M.G. Effects of acute nicotine on several operant behaviors in rats, Pharmacology Biochemistry and Behavior, Accepted: 8/10/99. **(E0691401)**

27. Scallet, A.C. Detecting neurotoxic damage to the circumventricular organs (CVOS): susceptible brain tissues located outside the blood-brain barrier. Site Specific Neurotoxicity, Accepted: 7/1/99. **(E0212315)**

28. Scallet, A.C. Estrogens: neuroprotective or neurotoxic? Annals of the New York Academy of Sciences, Accepted: 7/20/99. **(E0212315)**

29. Schmued, L.C. and Hopkins, K.J. Fluoro-Jade and Fluoro-Jade B, Toxilogic Pathology, Accepted: 7/1/99. **(E0701301)**

30. Schmued, L.C. and Slikker, W. Black-gold: A simple, high resolution histochemical label for normal and pathological myelin in brain tissue sections. Brain Research, 837(1-2):289-297, 1999. Accepted: 4/6/99. **(E0701301)**

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33. Slikker, W. Risk assessment and neurotoxicology: Neurotoxicology in current protocols in toxicology. Current Protocols in Toxicology, Accepted: 7/8/99. **(NA)**

34. Slikker, W. Site-selective action. Cellular and Molecular Mechanisms of Toxin Actions, Vol. 4 Site-Selective Neurotoxicity, Accepted: 7/1/99. **(E0693001)**

35. Slikker, W. and Gaylor, D.W. Biologically-based dose-response model for neurotoxicity risk assessment. Toxicology Letters, 102-103:429-433, 1999. Accepted: 10/1/98. **(E0693001)**

36. Slikker, W., Youdim, M., Palmer, E., Hall, E., Williams, C. and Trembly, B. The future of neuroprotection. Annals of the New York Academy of Sciences, Accepted: 7/30/99. **(NA)**

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40. Yu, X., Imam, S.Z., Newport, G.D., Slikker, W. and Ali, S.F. Ibogaine blocked methamphetmamine-induced hyperthermia and induction of heat shock protein in mice. Brain Research, Accepted: 7/15/99. **(E0698301)**

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CHEMISTRY

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FreshTag™, an invention of Chemistry staff scientists, shown here in a package of shrimp, will monitor seafood decomposition by a color indicator.

INTRODUCTION

The Division of Chemistry provides specialized expertise in analytical chemistry to support NCTR research initiatives and the conduct of fundamental and applied research projects in support of the FDA. The strategic goals of the Division are: 1) the rapid development and modification of complex analytical techniques required to conduct research initiatives in collaboration with the eight research divisions of the NCTR; 2) provide quality control and surveillance analysis of animal feed and bedding, test agents and dosed feed; 3) the creation, development, or modification of instruments and methods to facilitate the measurement of analyte levels in complex matrices encountered in biological samples and foods and other FDA-regulated products; and 4) to respond to special initiatives in support of FDA's regulatory and enforcement programs. These goals are realized by a team that includes both support and research chemists.

Overview of the Chemistry Analytical Program

The analytical program includes three general areas of responsibility: a) development/modification of Good Laboratory Practices (GLP)-directed analytical techniques and assessments to certify dose levels of analytes in water, gavage solutions and complex matrices of animal diets; b) continuous quality control analysis of animal bedding and food monitoring levels of key nutrients or disqualifying contaminants; and c) development and monitoring of validation trials for FDA regulatory and enforcement programs.

Development of risk assessments, setting of regulatory standards as well as enforcement of regulations requires reliable, validated analytical procedures to underpin the conclusions of studies or withstand legal challenges. The analytical team combines the expertise of traditional bench chemists with division research scientists employing high performance technologies to develop cost-effective methods for determination and confirmation analysis of a variety of analytes.

The analytical effort also includes a chemical custodian working within the framework of Good Laboratory Practices. The custodian documents the acquisition, storage and weight certification for all compounds used for the preparation of doses for studies. Another critical aspect of the team is the continued surveillance of all purchased feed

and bedding lots, and water supplies for trace contaminants including fumonisin B_1 that would compromise the scientific studies at the NCTR. All rodent diets are routinely analyzed for essential nutrient content and for compliance to limitations on fumonisin, pesticide and trace metal content before they are accepted for studies. Wastewater is monitored to insure that Arkansas Pollution Control & Ecology permit criteria are met for overall quality including levels of dissolved metals.

ONGOING ANALYTICAL CHEMISTRY AND FY1999 ACCOMPLISHMENTS

The NIEHS/NCTR National Toxicology Program (NTP) IAG represents the major workload commitment for the analytical program. Division chemists provide chemical characterization and purity of test compounds and customize or develop new methods for detection of test analytes at the dose levels, and in the matrices, mandated by the experimental design. Upon initiation of studies, the program evaluates dose homogeneity, stability and dose-level certification for the experiments. Studies for chloral hydrate were completed; ethinyl estradiol, genistein, leucomalachite green, malachite green, methoxychlor, nonylphenol and ethanol/urethane studies were initiated. Analyses were conducted in compliance with applicable GLPs related to maintenance of chemical custodial logs, chain-of-custody records and experimental raw data. Chemistry summary reports were prepared or are in preparation, for inclusion in final reports, for all the IAG-supported studies.

Instrumental techniques are customized to address specific analytical requirements: e.g., chloral hydrate levels were determined in gavage solutions by gas chromatography-flame ionization detection (GC-FID) with a minimum detection limit (MDL) of 2.0 µg/ml; ethinyl estradiol levels were determined in custom, soy-free rodent diet (NIH-31C; *i.e.,* Purina 5K96) by capillary GC-mass spectrometry (MS) (MDL of 0.2 ng/g); genistein levels were determined in the soy-free diet by high performance liquid chromatography (HPLC)-fluorescence (MDL of 0.5 µg/g diet); leucomalachite green levels were determined by HPLC- fluorescence (MDL of 1.0 µg/g rodent diet; *i.e.,* NIH-31); malachite green levels were determined by HPLC-Vis (MDL of 50 ng/g rodent diet); methoxychlor levels were determined by capillary GC-electron capture (EC) (MDL of 20 ng/g soy-free diet); nonylphenol levels were determined by HPLC-fluorescence (MDL of 1.0 µg/g soy-free diet); and urethane levels were determined in dosing solutions using GC-FID (MDL of 1.0 µg/ml).

Further collaboration included isolation, and purification of genistein and related metabolites and identification of chloral hydrate and its metabolites from rat blood.

Participation in the validation process for regulatory methods. In collaboration with the Center for Veterinary Medicine (CVM) and Stuttgard National Agricultural Research Center (SNARC), division chemists provided expertise and analytical methods for determination of sulfonamide levels in fish tissues to help validate regulatory methods of analysis through FDA method trials. Chemistry also continued efforts to provide regulatory methods of analysis for amoxicillin, erythromycin A and lincomycin in aquaculture species and other edible tissues of concern to CVM.

Future Analytical Programs

- Development of methods of analysis and quality control analysis for the NIEHS/NCTR National Toxicology Program will be a continued priority of the Analytical Program. Several test agents under discussion (*e.g.*, herbal preparations, natural products and antibiotics) will present unique challenges in comparison to the single chemical entities now on test. It is expected that chemical characterization of these complex mixtures will lead to the controlled analysis of a single, critical component

- Continued specialized spectrometry support to allow structural elucidation of toxicants, metabolites and by-products associated with toxicology research at NCTR.

- Continued work towards official methods status for erythromycin.

Overview of Chemistry Research

Research scientists in the Division of Chemistry serve as principal investigators for fundamental and applied research studies and provide consultation and support services to the analytical program and other research programs at the NCTR. The strategic goals of Chemistry Research are:

1. provide consultative services and specialized support to the analytical program and research programs at the NCTR and the FDA;

2. develop new technologies to fingerprint analytes as diverse as bacteria and generic drugs in the presence of complex matrices;

3. create and develop novel technologies to monitor food quality and detect bacterial contamination;

4. develop computational models using pattern recognition and spectrometry for quantitative structural activity models;

5. nutrient modulation of bioassay results; and

6. characterization of herbal preparations.

Specialized Spectrometry Laboratories

Three specialized spectrometry facilities in the Division of Chemistry provide multiple strategies for structure elucidation and compound identification as well as research-grade instrumentation for metals analysis.

The mass spectrometry group provides a large service program for all investigators using standard probe and flow-injection interface techniques including electron-impact, chemical-ionization, or electrospray-ionization mass spectrometry. In addition, the laboratory provides non-routine ionization methods (including matrix-assisted laser desorption ionization (MALDI) or atmospheric pressure chemical ionization), other sample introduction techniques (such as GC, liquid chromatography (LC), or pyrolysis mass spectrometry), and tandem mass spectrometry. Quantitative analyses, (isotope dilution mass spectrometry) methods also can be arranged. The laboratory includes a Finnigan TSQ-700 (GC/MS), a Finnigan Voyager (benchtop GC/MS), a Hewlett Packard 5890 (GC/LC/MS), a Finnigan TSQ 7000 (LC/MS), and a Kratos MALDI 1 (benchtop MALDI/MS). In addition to these standard instruments, three prototype or modified instruments are available in the laboratory. These include Pyrolysis/MAB and chemical reaction interface mass spectrometry (CRIMS) systems based on quadrupole mass filters and a Vestec linear time-of-flight system using a high power Nd/YAG laser.

This year the mass spectrometry group is scheduled to move into newly completed laboratories as part of a new NCTR/Office of Regulatory Affairs (ORA) Mass Spectrometry Resource. Division scientists initially will represent the largest component of this planned facility. They will continue their support of division-, NCTR-, and ORA/Center-sponsored research efforts.

The Nuclear Magnetic Resonance (NMR) spectrometry group also provides a service function for scientists both within and outside the Division and Center. Routine analyses of "walk in" samples includes ^{1}H 1D, ^{1}H 1D homo-decoupling, ^{1}H 1D NOE-difference, ^{13}C 1D. Non-routine methods include ^{1}H 2D Nuclear Overhauser Effect Spectroscopy (NOESY), ^{1}H 2D Correlation Spectroscopy (COSY), ^{1}H 2D Rotating Overhauser Effect Spectroscopy (ROESY), ^{1}H 2D Total Correlation Spectroscopy (TOCSY), and 2D ^{1}H-^{13}C Hetero-correlation spectroscopy. The laboratory includes a Varian 300 MHz NMR, Bruker 500 MHz Aspect 3000 NMR, and Bruker Aspect 1000 data station. The probes on the Bruker 500 MHz have ^{1}H and ^{13}C probe capabilities. The probe on the Varian 300 MHz has ^{1}H, ^{13}C, ^{15}N, ^{19}F, and ^{31}P capabilities.

The laboratory specializes in "secondary" structure elucidation, chemical shift assignment, compound identity, and purity of samples. Both NMRs can be used for these purposes but the Bruker 500 MHz NMR has a better resolution and S/N ratio than the 300 MHz NMR and is better suited for larger compounds and dilute sample concentrations.

The metals analysis facility provides a clean room environment for several research grade instruments. Trace metal analysis can be accomplished with a variety of instrumental techniques. The TJA AA-Scan 4 graphite furnace atomic absorption spectrophotometer (AAS) provides simultaneous analysis of up to four elements simultaneously in the ppb range. The TJA PolyScan 61E inductively-coupled plasma emission spectrometer (ICP-AES) can detect almost any element in the periodic table at levels in the ppm range. Lastly, the Fissons PlasmaQuad XR inductively-coupled plasma mass spectrometer (ICP-MS), like the ICP-AES, can detect almost any element

in the periodic table, however, the limits of detection are lowered to the parts-per-trillion range.

ONGOING PROJECTS AND FY1999 RESEARCH ACCOMPLISHMENTS

FreshTag™ is a consumer-based, low-tech indicator of food freshness. Division scientists patented, licensed, and developed for manufacture, a system of test strips for food decomposition called **FreshTag™**. Product configurations suitable for commercial manufacture have been developed. Most of the equipment needed to manufacture over 10 million tags per year has been tested and proved. This product will address the needs of inspectors and buyers for rapid testing of bulk seafood products. A configuration suited for inclusion within retail packages is being developed in collaboration with Cox Recorders of Belmont, NC, the licensee. The goal is to produce the tags on an engineered film that can be produced on a scale of about one million tags per day. By monitoring biogenic amines, the product is an indirect measure of food decomposition and provides the consumer with some of the benefits of having their own, highly-trained FDA organoleptic expert. **FreshTag™** and its inventors Dwight W. Miller, Ph.D. and Jon G. Wilkes, Ph.D. were recipients of the 1999 Popular Science Award "Best of What's New."

The mass spectrometry group applies a significant research effort to food quality applications. These include:

1. development of pyrolysis mass spectrometry and pattern recognition technology for rapid assessment of patterns that can characterize complex mixtures and trace contaminants in bulk drugs;

2. development of pyrolysis and matrix assisted laser desorption ionization (MALDI) mass spectrometry methods for the rapid detection of bacteria cultured or directly collected from contaminated products; and

3. development of an in-line chromatographic solvent removal interface (universal interface) that can adapt HPLC separations to several mass spectrometric or other gas phase modes of detection to permit drug metabolism analysis without utilizing radiotracer methods.

Pyrolysis mass spectrophotometric methods using pattern recognition for comparing and classifying complex chemical mixtures have continued to be examined for their utility. For example, instrumentation developed in the NCTR mass spectrometry laboratory has been demonstrated for quality control of bulk drug ingredients. The technique can classify bulk chemical batches by their manufacturer, or production lot, and rapidly identify ions associated with trace impurities that distinguish the samples; a "fingerprint analysis." This approach permits rapid screening (by FDA scientists, for example, as a substitute for off-shore inspections of disbursed small-scale plants) of imported raw ingredients used by domestic manufacturers in product formulation. The technique also can be used to detect adulterated foods or cosmetics, or to differentiate generic preparations from brand name pharmaceutical products.

Mass spectrometry methods for the detection of bacteria generally rely on chemotaxonomy, the characterization of the organisms based on differences in chemical composition. Pyrolysis mass spectrometry samples the chemical composition of bacteria by thermally producing volatile, small molecules as the bacteria decompose during rapid heating. By comparison, the mass spectrometry technique of matrix assisted laser desorption ionization (MALDI MS) rely on relatively large molecules and even intact proteins. In MALDI MS, the laser heats the sample so rapidly that the large molecules are desorbed directly from the surface, including the surface of untreated cells. Both pyrolysis and MALDI techniques can produce a spectral fingerprint analysis of bacteria including unique genus-, species-, or property-specific biomarkers.

Using MALDI, the identity of two acid-resistance biomarkers, products of the rpoS genes were established from the mass spectrum of intact *E.coli* and *Shigella flexneri*. These same biomarker proteins also have been detected directly in samples taken from contaminated water, vegetables, and cotton cloth. **These identifying spectra were obtained without the time-consuming step of multiplying the bacterium with culture techniques.** In another demonstration of the techniques, both MALDI/TOF and pyrolysis mass spectrometry, combined with the pattern recognition, successfully identified several taxonomically similar, but toxicologically distinct strains of *Vibrio parahaemolyticus* supplied by the Center for Food Safety and Applied Nutrition (CFSAN) scientists.

Cooperative Research and Development Agreement (CRADA) to develop a universal interface for mass spectrometry analysis of HPLC streams. The volume and complexity of solvents has hampered the marriage of resolving power of HPLC and mass spectrometry. This interface, coupled with chemical reaction interface mass spectrometry (CRIMS), would facilitate drug and toxin metabolism studies without using radioisotopes as tracers; *e.g.*, HPLC/CRIMS is a critical interface. Research has continued on development of an improved universal interface between HPLC and mass spectrophotometers to obviate this problem. Prototypes for the two stages of the redesigned device have been received from Scientific Instruments Services, Inc. (SIS), the CRADA partner supporting this research. The stages have been separately characterized for their solvent removal capabilities and other performance features compared to theoretical models.

The NMR and mass spectrometry groups, using computational techniques and spectrometry, developed quantitative structural activity relationship (QSAR) models for rapidly identifying interaction characteristics of chemicals with biological systems. The method uses the pattern recognition capabilities previously developed for use with mass spectrophotometry. This new initiative, however, extends the technique to evaluate patterns in composite spectra of the chemicals from different spectrophotometric endpoints including the NMR. Based on an extensive training set, it establishes the relationship between composite spectral patterns and biological endpoints. The new system is extraordinarily rapid and flexible compared to all previous art. These

techniques are expected to provide rapid and relatively inexpensive supplements or alternatives to *in vivo* studies.

Nutritional modulation of bioassay results. It is well established that in rodents, high rates of body growth in early life results in increased incidence of spontaneous neoplasms and reduced survival. This NTP-funded study used controlled feeding to manipulate body growth in male $B_6C_3F_1$ mice that were treated with the sedative drug, chloral hydrate as part of a two-year cancer bioassay of this compound. The controlled feeding (CF) was designed to adjust the body weights of the mice to fit an idealized weight curve for $B_6C_3F_1$ mice, which predicts a terminal, background liver-tumor incidence of 15%; these mice were compared to *ad libitum*-fed (AL) counterparts.

During 1999 the full pathological analysis of the mice from the two-year studies was completed as was the biochemical and pharmacokinetic analysis of the mice, acutely-treated with chloral hydrate. This approach successfully maintained cohorts of mice at weights approximating their idealized target weights throughout the two-year study. Chloral hydrate increased terminal liver tumor incidence in both the AL and CF diet groups, but a statistically significant dose-response was observed only in the CF mice. This observed increase in assay sensitivity did not appear to be due to diet effects on pharmacokinetics since there was no significant difference in the clearance (area under the curve) of the compound or its major metabolite between the CF and AL mice. A technical report for this study is in preparation.

Characterization of herbal preparations. Medicinal herbs have been used throughout history and there is little question some have preventative and/or therapeutic effects; many modern drugs are variants of compounds isolated from herbal sources. With a recent rise in popularity, the use of herbs has risen and as with any medicine, some individuals are more sensitive than others to both therapeutic and adverse effects. There is high variability in the quality of herbal preparations and little knowledge of the principal components of these preparations or of the therapeutic or toxic components. Five common herbal products have been selected for characterization, including St. John's Wort (*Hypericum perforatum*), *Ginkgo biloba*, turmeric (*Curcuma longa*), goldenseal (*Hydrastis canadensis*), and *Aristolochia*. Conditions for the analysis of the principal components of St. John's Wort and evaluation of five brands have been completed.

Although hypericum is commonly associated as the active component of St. John's Wort, at least ten components may contribute to its pharmacological action(s). Extraction with acetone at 55 degrees was optimal in comparison with sonication techniques and reverse-phase HPLC conditions were developed to separate and quantify all ten components in a single pass. The concentrations of individual components, respectively, varied between approximately two- to five-fold among the five preparations; hypericin varied four-fold. The variation in the components appears to be independent of the brand suggesting that factors other than "purity" of the herbal preparation may be causing this difference.

FY2000 GOALS

- Extension of the FreshTag™ concept to include other food products and other indicators of food quality. In addition, new devices will be developed for quantitative measurement of decomposition or adulteration of products.

- Continued development of MALDI TOF mass spectrometry, along with the complementary pyrolysis method for bacterial characterization, to characterize samples associated with pathogens in seafood. Preliminary samples obtained from FDA's CFSAN facility in Florida have suggested that these methods may allow bacteria associated with human clinical outbreaks to be correlated with the correct strains from a reference collection of bacteria.

- Complete testing, design, and finalize performance characteristics for the Universal Interface. The CRADA partner will evaluate the feasibility of mass producing the redesigned interface for HLPC-mass spectrometry, HPLC-infrared (IR), HPLC-CRIMS, and other applications.

- Computational Science. Automation of the pattern recognition procedure for the process of identifying bacteria based on spectral patterns.

    - The spectral activity computational model will be evaluated for other biological endpoints including carcinogenicity, mutagenicity, and additional protein binding relationships.

    - A model will be developed using quantum mechanics and artificial neural networks for predicting the log of LD_{50} values.

- Study the effects of selected herbal preparations on prescription drugs.

- Define the major components of *Angelic sinensis* root, the traditional Chinese herbal known as *Dong quai,* that is used to treat gynecological diseases.

PUBLIC HEALTH SIGNIFICANCE

The development and validation of relevant analytical methods will enable Center scientists and the Agency to perform analyses of food, drug, and cosmetic products for constituents that the FDA has responsibility for regulating in order to make rapid decisions on the disposition of the products requiring action.

The development of rapid and inexpensive analytical methods to determine food quality will enable the Agency, food broker, and consumer to perform quality determinations of food in order to make rapid decisions on the disposition of the food products.

The development of computational techniques to predict toxicological endpoints will enable the Agency and toxicologists to evaluate new and existing products for these safety parameters at a reduced cost.

ACTIVE PROJECTS FY1999

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0211701	**Chronic Bioassay of Chloral Hydrate in Male $B_6C_3F_1$ Mice Using Idealized Body Weight Curves that are Normalized by Modulation of Caloric Intake** To determine the chronic toxicity and potential carcinogenicity of chloral hydrate administered by aqueous gavage, to male $B_6C_3F_1$ mice.To determine the feasibility of utilizing dietary control (i.e., the manipulation of caloric intake) to control body weight gain so that all mice in each experimental group of the bioassay conform to an idealized weight curve.	Leakey, Julian E.* Agrawal, Nalini Contrera, Joseph Turturro, Angelo
E0211711	**ADDEND: Chronic Bioassay of Chloral Hydrate in Male $B_6C_3F_1$ Mice using Idealized Body Weight Curves that are Normalized by Modulation of Caloric Intake** Additional animals requested for training purposes in troubleshooting/optimizing procedures for blood collection and extraction of chloral hydrate from serum.	Leakey, Julian E.* Seng, John E.
E0211722	**ADDEND: Dose Response to Chloral Hydrate in Dietary Restricted Mice** To determine the effect of two levels of dietary restriction on the pharmacokinetics, metabolism and acute hepatotoxicity of chloral hydrate in male $B_6C_3F_1$ mice.	Leakey, Julian E.* Seng, John E.
E0260201	**Effect of Caloric Restriction on Rat Testicular Tumor Formation** All of the aims of this proposal are directed towards understanding the role of dietary components (i.e., caloric restriction) in influencing the ultimate susceptibility of the male reproductive tract to chemical insult.	Leakey, Julian E.* Gandy, Jay Manjgaladse, Mikhail
E0260211	**ADDEND: Effects of Caloric Restriction on Rat Testicular Tumor Formation** Addendum outlines additional work required to aid in the interpretation of the data obtained in the master project (E02602.01). Propose to determine macrophage concentrations in the tissue samples collected as part of E02602.01. Plan to acutely expose rats to flutamide for two wks and then determine testicular malondialdehyde concentrations. Animal work will be performed in Dr. Gandy's lab at UAMS – requests that NCTR provide NIH 31 diet and provide histopathological evaluation. Additional path hours projected and research hours.	Leakey, Julian E.* Gandy, Jay Seng, John E.
E0260221	**Effects of Caloric Restriction on Rat Testicular Tumor Formation - Addendum, Collection and Analysis of Additional Tissues** To further investigate the effects of caloric restriction, aging and circadian time-point on testicular lipocortin 1 levels. This work requires alterations in the way rats that are scheduled to be culled will be sacrificed.	Leakey, Julian E.* Gandy, Jay Seng, John E.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0687401	**Development of Devices/Methods for Determination of Food/Seafood Quality** Assist FDA with problems incurred in testing seafood for decomposition by developing an expeditious assay for determining volatile and semivolatile organic compounds in spoiled seafood.	Miller, Dwight W.* Freeman, James P. Grahn, Meredith Heinze, Thomas M.
E0693601	**Development of Analytical Methods for Determination of Amoxicillin and Lincomycin in Fish Tissues** Develop highly sensitive analytical methods utilizing reversed-phase HPLC or GC for determining trace levels of amoxicillin and lincomycin residues in fish tissues. Specifically, the goal is to develop analytical methods which can be applied to determine amoxicillin in catfish muscle tissue and salmon muscle and skin tissues at 10 ppb and to determine lincomycin in salmon muscle and skin tissues at 100 ppb as suggested by the FDA Center for Veterinary Medicine (CVM). Separate procedures/solvent systems for the extraction, cleanup and HPLC analysis of each antibiotic are expected to be necessary because of the structural differences between amoxicillin and lincomycin. However, analytical residues in both the catfish and salmon tissue substrates will be developed if feasible.	Ang, Catharina Y.* Freeman, James P. Hansen, Eugene Luo, Wenhong Settepani, Joseph
E0693611	**ADDEND: Development of Analytical Methods for the Determination of Amoxicillin and Lincomycin in Fish Tissues and B-Lactam Antibiotics in Bovine Milk** Expand the original protocol to include another substrate, bovine milk, and to include the determination of four additional B-lactam antibiotics in milk. Also requesting 6-month extension of project.	Ang, Catharina Y.* Churchwell, Mona I. Doerge, Daniel R. Hansen, Eugene Luo, Wenhong Thompson, Harold C. Settepani, Joseph
E0693801	**Quantitative Determination of Enantiomers Composition and Purity of Drugs by Nuclear Magnetic Resonance (NMR) Spectroscopy** 1. To develop NMR methods to monitor enantiomeric purity of a group of Beta-adrenergic antagonists (i.e., propranolol, sotalol, pindolol and timolol.) The hypothesis is that effective NMR methods can be developed to monitor the enantiomeric purity of these drugs. 2. To develop NMR methods to monitor degradation products of a coronary vasodilator (nifedipine). The hypothesis is that effective NMR methods can be developed to monitor the degradation products of this drug.	Evans, Frederick E.* Hanna, George M.
E0698001	**Development of Methods for Analysis and Confirmation of Erythromycin A Residues in Tissue Samples from Terrestrial and Aquatic Farmed Animals by Liquid Chromatography** The principal objective of this project is to develop determinative and confirmatory analytical chemical procedures, using high performance liquid chromatography/electrochemical detection and high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometric detection, for Erythromycin A in biological samples taken from agricultural animals. Specifically, the goal is to develop complete methods for the analysis of Erythromycin A in muscle and liver tissues from poultry, non-processed bovine milk, and muscle tissues from salmon, catfish and shrimp. Sensitivity levels for these methods are expected to be at least 100 parts per billion for liver tissue and 50 parts per billion for muscle tissue and milk as requested by the Center for Veterinary Medicine.	Hansen, Eugene* Ang, Catharina Y. Churchwell, Mona I. Doerge, Daniel R. Luo, Wenhong Wilkes, Jon G. Alderson, Norris

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0698901	**Development of Methods for Analysis of Volatile and Nonvolatile N-Nitrosamines in Relevant Cosmetics and Nitrite Cured Meat Products** Develop methods for extraction, cleanup, and analysis of non-volatile N-nitrosamines in cosmetics and meat products using combined liquid chromatography (LC) detection methods with confirmation by compatible mass spectrometry (MS) ionization methods; Investigate the applicability of Liquid Chromatography-electron spray ionization/mass spectroscopy (LC-ESI/MS) and/or (LC-APCI/MS) as a multiresidue, trace level, quantitative technique for analysis of volatile, semi-volatile, and non-volatile N-nitrosamines in these consumer products.	Billedeau, Stanley M.* Churchwell, Mona I. Cooper, Willie M. Doerge, Daniel R. Wilkes, Jon G. Fiddler, Walter Pohland, Albert
E0700601	**Development of Multiresidue Methods to Determine and Confirm Sulfonamides in Edible Tissues of Aquacultured Species** To develop analytical chemical methods to determine and confirm sulfonamide (SA) residues at the 1-10 ng/g level in edible tissues of aquacultured species. Technologies used will include liquid chromatography (LC) with postcolumn derivatization and fluorescence detection for the determinative procedure and liquid chromatography with atmospheric pressure chemical ionization mass spectrometry (LC-APCI/MS) for the confirmatory procedure.	Gehring, Theresa A.* Churchwell, Mona I. Cooper, Willie M. Doerge, Daniel R. Holcomb, Manuel Rushing, Larry G. Thompson, Harold C. Alderson, Norris
E0693101	**First Phase Development of a Rapid Screening Method for Identification of Complex Mixtures by Pyrolysis-Mass Spectrometry with Computerized Pattern** Evaluate feasibility of the application of pyrolysis mass spectrometry (PyMS) with computerized pattern recognition (PattRec) for the rapid identification of a sample (a) which is a complex chemical mixture, (b) which is member of a set of such mixtures, and (c) for which there is a regulatory need to distinguish the individual members of the set. Typical examples of applications: (a) the rapid identification of culturable pathogenic and non-pathogenic bacteria in food, (b) the distinction of adulterated from pure foods or cosmetics, or of generic from brand name pharmaceutical products, or (c) demonstrating the virginity of plastic materials used in food containers.	Wilkes, Jon G.* Chen, James J. Fry, Fred Heinze, Thomas M. Kaysner, Charles A. Lay, Jackson O. Miller, Dwight W. Rafii, Fatemeh Sutherland, John B. Turturro, Angelo Voorhees, Kent J.
E0697201	**Universal Interface Development and Applications** The ultimate objective of this work is to develop, if possible and practical, a variety of new technologies for improving high performance liquid chromatography (HPLC) detection. By eliminating hazards associated with radioactivity, it can make possible metabolic drug studies involving human subjects. Several CRADAs will be negotiated during the work to facilitate development of commercial versions of the devices which show the most promise.	Wilkes, Jon G.* Abramson, Fred Billedeau, Stanley M. Freeman, James P. Heinze, Thomas M. Pothuluri, Jairaj V.
E0699701	**Innovative Methods for Determining Food Quality: Decomposition, Safety and/or Economic Fraud** Examination of the total volatile bases (TVB) and putrescine (PU), cadaverine (CD) and histamine (HS) methods for potential regulatory use and validation of TVB as an indicator of decomposition; Develop rapid detection methods for the determination of decomposition analytes in seafood.	Miller, Dwight W.* Freeman, James P. Heinze, Thomas M. Holcomb, Manuel Lansden, John A. Lay, Jackson O. Wilkes, Jon G.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0700501	**Rapid Identification of Intact Whole Bacteria Based on Spectral Patterns Using Matric Assisted Laser Desorption Ionization Time-of-Flight Mass** 1. To evaluate the potential use of matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) as a method for the rapid identification of whole bacteria, either by comparison with archived reference spectra or by co-analysis with cultures of known bacteria; 2. To establish a standard set of conditions for the acquisition of MALDI/TOF mass spectra from bacteria suitable for use in bacterial identification; 3. To obtain some measure of the distribution of signals (ions at specific masses) obtained using standard MALDI/TOF MS conditions based on the analysis of a variety of related and unrelated bacteria; 4. To use standard (pattern recognition) as well as newer (artificial intelligence and principal components analysis) mass spectral recognition techniques to evaluate whether or not the standardized mass spectra obtained from bacteria are sufficiently distinct to allow identification of specific bacteria or to select related bacteria from a group; 5. To evaluate the use of mass spectral recognition techniques for the identification of bacteria from mixtures based on MALDI/TOF MS analysis of the mixture; 6. To determine the minimum number of bacteria necessary for obtaining standard mass spectra; 7. To evaluate the effects on the reproducibility of spectra obtained from whole bacteria under different conditions of sample handling, storage, and cell growth.	Lay, Jackson O.* Darsey, Jerry Heinze, Thomas M. Holland, Ricky D. Miller, Dwight W. Muser, Steven M. Rafii, Fatemeh Sutherland, John B. Voorhees, Kent J. Wilkes, Jon G.

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2. Beland, F.A., Doerge, D.R., Churchwell, M.I., Poirier, M., Schoket, B. and Marques, M. Synthesis, characterization, and quantitation of a 4-aminobiphenyl DNA adduct standard. Chemical Research in Toxicology, 12:68-77, 1999. Accepted: 11/4/98. **(Collaborating with Biochem. Tox.) (S00198)**

3. Billedeau, S.M., Holcomb, M. and Wilkes, J.G. Comparison of MAB vs EI ionization for MS analysis of N-nitrosamines and similar compounds. Proceedings of the American Society of Mass Spectrometry, 47:118, 1999. Accepted: 6/14/99. **(E0693101)**

4. Chang, C., Holland, R.D., Churchwell, M.I. and Doerge, D.R. Inactivation of peroxidase by 4-chloroaniline during turnover: Comparison with horseradish peroxidase and bovine lactoperoxidase. Chemico-Biological Interaction, 123:197-217, 1999. Accepted: 8/15/99. **(Collaborating with Biochem. Tox.) (E0692001)**

5. Doerge, D.R., Churchwell, M.I., Gehring, T.A., Pu, Y. and Plakas, S. Analysis of malachite green and metabolites in fish using LC-APCI/MS. Rapid Communications in Mass Spectrometry, 12(21):1625-1634, 1998. Accepted: 10/1/98. **(E0211901)**

✳ **(_ _ _ _ _ _ _ _)** ***Indicates the related NCTR project number; NA-Not Applicable***

6. Duhart, B.T., Zhang, D., Moody, J.D., Freeman, J.P. and Cerniglia, C.E. Biotransformation of protriptyline by filamentous fungi and yeasts. Xenobiotica, 29:733-746, 1999. Accepted: 7/16/99. **(Collaborating with Microbiology) (E0694201)**

7. Hart, R.W., Bucci, T.J., Turturro, A., Leakey, J.E., Feuers, R.J., Duffy, P.H., James, S.J., Lyn-Cook, B.A., Pipkin, J.L., Li, S. Caloric intake as a modulator of carcinogenicity and anticarcinogenicity. Carcinogenic/Anticarcinogenic factors in food: Novel Concepts, Accepted: 3/12/99. **(Collaborating with Ofc. of Dep. Dir.) (EO260112)**

8. Hart, R.W., Duffy, P.H., Fu, P.P., Leakey, J.E., Seng, J.E., Turturro, A. and Li, S. The interaction of energy metabolism with xenobiotic pathways. In: Energy Metabolism and Carcinogenesis, Accepted: 5/1/99. **(Collaborating with Ofc. of Dir./Imm. Ofc.) (E0699801)**

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10. Holder, C.L., Churchwell, M.L. and Doerge, D.R. Quantification of soy isoflavone, genestein and daidzein and conjugates in rat blood using LC/ES-MS. Journal of Agricultural and Food Chemistry, 47(9):3764-3770, 1999. Accepted: 7/12/99. **(NA)**

11. Holland, R.D., Duffy, C.R., Rafii, F., Sutherland, J.B., Heinze, T.M., Holder, C.L., Voorhees, K., Lay, J.O. Identification of bacterial proteins observed in MALDI TOF mass spectra from whole cells. Analytical Chemistry, 71:3226-3230, 1999. Accepted: 5/5/99. **(E0700501)**

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GENETIC AND REPRODUCTIVE TOXICOLOGY

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GENETIC TOXICOLOGY LABORATORY

INTRODUCTION

Research support scientist, Bo Mittelstaedt, uses an automated DNA sequencer to determine if mutations have occurred in the H*prt* gene of lymphocytes.

The FDA requires that petitioners provide data evaluating the potential genetic toxicity of food additives, human and animal drugs, and biological therapies as part of the product approval process. As part of the regulatory function of the FDA, it is necessary to identify and measure the potency of suspected mutagens and carcinogens. Regulatory decisions are based not only on the identification of potentially hazardous genotoxic and putative non-genotoxic substances, but also on an understanding of their modes of action. In order to address these needs, the Genetic Toxicology Laboratory conducts fundamental research aimed at defining the pathways from initial DNA damage to mutation. Research within the Genetic Toxicology Laboratory centers on the development and validation of new methodologies by which to assess genetic risk.

The focus of this laboratory is on the development and validation of *in vivo* mammalian systems to measure spontaneous and induced somatic mutations. Further, the laboratory has made substantial progress in detecting the broad spectrum of events that occur during the carcinogenesis process. An increased understanding of mutational mechanisms, combined with test systems with an increased capability to detect genetic damage, will provide the regulatory process with the most current knowledge on which to base regulatory decisions. In order to ensure that the regulatory process is based upon the most current understanding of mutational mechanisms, the goal of this laboratory is to develop, validate, and implement reliable and sensitive mutation detection systems.

FY1999 ACCOMPLISHMENTS AND FY2000 PLANS

Early in FY1999, the Genetic Toxicology Laboratory was reviewed by the Genetic Toxicology site visit team of the NCTR Science Advisory Board. The scope and theme of the Laboratory were presented by the Division Director and were followed by the individual research programs of the senior members of the Laboratory. The

development, validation, and implementation of the rat lymphocyte *hypoxanthine-quanine phosphoribosyl transferase (Hprt)* assay were considered to be significant achievements that have led to the recognition of the Laboratory as a premiere authority in this field. Also considered to be highly meritorious research programs were the validation of the Big Blue Transgenic Rat Assay and studies which addressed the role of programmed cell death in mutation. The creation of a transgenic mouse, heterozygous at the thymidine kinase locus, and the ability to detect mutations induced by known carcinogens in splenic lymphocytes from this animal, were considered to be outstanding contributions to the study of *in vivo* mutagenesis. A new project, in which methods for genotypic selection were implemented to measure rare mutational events at the H-ras locus in carcinogen-exposed mice, received excellent comments. The Laboratory received an outstanding review from the site visit team.

The Laboratory has made significant progress toward its goals of developing, validating, and implementing sensitive *in vitro* and *in vivo* systems to detect mutational events. In the *in vitro* component, a series of experiments was conducted to assess the *p53* functional status of human lymphoblastoid cells that are heterozygous at the *Thymidine kinase (Tk)* locus. Molecular genetic techniques revealed that a mutation existed in the *p53* gene of AHH-1 $Tk^{+/-}$ in contrast to the wild-type sequence found in the companion cell line, L3. Exposure of these cell lines to compounds of interest to the FDA results in a higher mutant frequency in AHH-1 $Tk^{+/-}$ than in L3. This suggests that *p53* functional status is a factor in determining the mutant frequency at the *Thymidine kinase* locus.

The rat lymphocyte *Hprt* assay has been employed extensively in experimentation within the Laboratory. In a series of experiments that addressed the role of anti-oxidants in chemically induced mutation, the treatment of mutagen-exposed rats with vitamin supplements reduced the mutant frequency in splenic lymphoctyes. In addition, dietary restriction was shown to reduce both the spontaneous and bleomycin-induced *Hprt* mutant frequencies in both male and female rats. The rat lymphocyte assay was also utilized to determine the spontaneous mutant frequency at the *Hprt* locus in the Big Blue Rat for comparison to the spontaneous mutant frequency in the transgenes, *lacI* and *cII*. In a collaborative study with CFSAN, an additive increase in the *Hprt* mutant frequency was demonstrated in rats intermittently fed the liver carcinogen, aflatoxin B_1.

Studies aimed at the validation of the Big Blue Rat Assay continued within the Laboratory. Specific experiments included: comparison of the mutant frequency between *lacI* and *Hprt* in splenic lymphocytes obtained from DMBA-, thiotepa- and 2-AAF-exposed Big Blue Rats; comparison of N-OH-2-AAF- and DMBA-induced *lacI* mutations in target and non-target tissues; comparisons of the mutation spectra in *lacI* and *Hprt* of DMBA-, thiotepa-, and N-OH-2AAF-exposed Sprague Dawley, Fischer 344, and Big Blue Rats; comparison of DMBA- and N-OH-2-AAF-induced DNA adducts in target and non-target tissues and correlation with *lacI* mutant frequencies; and the comparison of *cII* mutation frequencies with *lacI* mutation frequencies in target and non-target tissues. These experiments have led to the recognition that the Big Blue Rat is a useful model for detecting tissue-specific mutational events.

A second transgenic model under investigation is the ΦX174 am3 mouse whose low spontaneous mutant frequency is advantageous in detecting weak mutagens. Studies with the "super mutagen", ethnylnitrose urea (ENU), indicate that the reversion assay may not possess the sensitivity necessary for mutagen screening. However, a forward assay using this system is under development and the results of initial experiments suggest that it will be sensitive for the identification of certain classes of mutagens.

A third transgenic model undergoing characterization in the Genetic Toxicology Laboratory is the $Tk^{+/-}$ mouse. This mouse was created by gene-targeting technology in embryonic stem cells in order to detect recombination, deletion, loss of heterozygosity, and intragenic mutation. Accomplishments to date include: derivation of the $Tk^{+/-}$ mouse; establishment of conditions for quantifying *Tk* lymphocyte mutants; and measurement of spontaneous, as well as ENU-, radiation-, and DMBA-induced mutant frequencies.

Methods development has been completed and experimentation is underway for two new initiatives, the development of genotypic selection techniques for the mouse *H-ras* gene and the analysis of aflatoxin B_1-responsive genes in primary cultured hepatocytes using differential display polymerase chain reaction (PCR) and differential hybridization of a high-density filter array. Genotypic selection techniques are being applied to the measurement of spontaneous and 4-aminobiphenyl-induced H-ras codon 61 mutations in livers of C57Bl6/J, $B_6C_3F_1$, and mismatch-repair deficient, Pms2 mice. Early studies on the modulation of gene expression in aflatoxin B_1-exposed human and rat hepatocytes have revealed the differential expression of genes involved in the toxic response to DNA damaging agents.

The work described here is directed towards understanding mechanism(s) somatic mutation induction in model *in vivo* systems. It is important to validate their usefulness for predicting the carcinogenicity of chemicals of interest to the FDA in order to provide the product review process with the most current and reliable data on which to base regulatory decisions. The methodologies range from quantification of mutation for genetic risk assessment to mutational spectra analysis for reliable and accurate cross-species comparison. The desire to develop highly sensitive techniques to detect mutagens whose primary mode of action does not include direct DNA adduction also is a primary motivation for the direction of the research. Included in the research activities are efforts to understand how mutagens influence the fixation of the altered sequence through modulation of gene expression and the role of the *p53* tumor suppressor gene in determining the recovery of chemically-induced mutations.

FY2000 GOALS

1. Develop and validate sensitive and predictive *in vitro* systems to identify, quantify and understand the mode of action of potential human toxicants, especially carcinogens and mutagens.

2. Develop and validate sensitive and predictive *in vivo* systems to identify, quantify and understand the mode of action of potential human toxicants, especially carcinogens and mutagens.

In order to achieve the first goal, the AHH-1 human lymphoblastoid system will be utilized to evaluate risk to the human genome. The cell lines, AHH-1 $Tk^{+/-}$ (mutant *p53*) and L3 (wild-type *p53*), differ in the spontaneous- and induced- mutant frequencies at the *Tk* locus. Molecular analysis of the induced mutants is currently underway in order to clarify the role of *p53* in determining the mutation spectra in the human genome.

The approach to the second goal is the continued use of transgenic and nontransgenic rats and mice in the evaluation of mutagens and carcinogens of interest to the FDA. These systems would complement the present *in vivo* rodent genotoxicity assays utilized by the FDA. As these systems are further refined, their increased sensitivity could lead to their inclusion in the test battery and aid the FDA in the product review process. These systems provide a means to evaluate damage to critical genes in the target organ as well as in surrogate tissue. In addition, they provide a means by which mechanistic questions can be asked in an *in vivo* system. Finally, they provide the ability to make critical evaluations and comparisons of molecular alterations in transgenes, reporter genes, and cancer genes. This will enable a more direct comparison to the molecular events described in human cancer.

PUBLIC HEALTH SIGNIFICANCE

Methods for the identification of and quantitative measurement of the potency of suspected carcinogens and mutagens are essential to the FDA regulatory process. The systems developed and characterized by the Genetic Toxicology Laboratory are capable of simulating human exposure, increasing the ability to detect weak carcinogens, and decreasing the time required for determining genotoxic potential. Additionally, the data generated will provide mechanistic information regarding the mode(s) of action of certain chemical classes. This will provide a more accurate and rapid assessment of the potential risk to the human population.

ACTIVE PROJECTS FY1999

Project Number	Title/Objective	Principal*/ Co-Principal Investigator(s)
E0685300	**Construction of Transgenic Hamster Ovary Cells Expressing Arylsulfotransferases (AST) IV and Their Use in Studies of Molecular Mechanism of Arylamine- and Polycyclic Aromatic Hydrocarbon-Induced Carcinogenesis** 1. To construct a mammalian expression vector containing the AST IV gene and to transvect Chinese hamster ovary cells with the recombinant vector; 2. To use these transgenic cells in the Hypoxanthine-guanine phosphoribosyl transferase (*Hprt*) and Adenosine phosphoribosyl transferase (A*prt*) mutation assays.	Yerokun, Tokunbo* Heflich, Robert H.
E0687801	**Evaluation of the Effects of Dietary Antioxidants on Lymphocyte Function and Genotoxicity Induced in Young and Old Rats Exposed to DNA-damaging Agents *In Vivo*** 1. To determine the effects of the antioxidant vitamins on the genotoxicity induced by exposing mutagens/carcinogens to young and old rats; 2. To determine the effects of antioxidant vitamins on lymphocyte function in mutagen-exposed and non-exposed young and old rats.	Lyn-Cook, Lascelles E.* Casciano, Daniel A. Wamer, Wayne
E0687811	**ADDEND: Evaluation of the Effects of Dietary Antioxidants on Lymphocyte Function and Genotoxicity Induced in Young and Old Rats Exposed to DNA-Damaging Gents *In Vivo*** Request for additional rats to complete project - 36 rats required for four additional batches of conditioned media; Investigator who was going to conduct Exp. #5 has left the Center; Exps. 2, 3 and 5 will be conducted separately; additional animals are requested to achieve this; sixteen rats are requested for each phase of each exp. - total of 228 additional animals requested.	Lyn-Cook, Lascelles E.* Aidoo, Anane Casciano, Daniel A. Wamer, Wayne
E0690601	**Quantitative and Molecular Analysis of 7,12-Dimethyl-benz[a]anthracene-(DMBA) induced mutations in the model Blue Rat: Comparison of Mutagenesis in the Transgene lacI with the Endogenous gene *Hprt* and Cancer Genes H-ras and P53** 1. To determine the mutant frequency and mutation spectrum of the *lacI* transgene of the Blue Rat following exposure to DMBA in surrogate and target tissues and compare these mutant frequencies and mutational spectra to those determined in Objectives 2 and 3; 2. To determine the mutant frequency and mutation spectrum of the endogenous *Hprt* reporter gene in T-lymphocytes from the spleens of Fisher 344 and Blue Rats following exposure to DMBA; 3. To induce mammary tumors in Fischer 344 rats and Blue Rats by exposure to DMBA and screen tumor DNA for mutations in the oncogene, H-ras and the tumor suppressor gene, p53.	Manjanatha, Mugimane* Aidoo, Anane Casciano, Daniel A. Heflich, Robert H. Lyn-Cook, Lascelles E. Mittelstaedt, R. A. Short, Jay M.

Project Number	Title/Objective	Principal*/ Co-Principal Investigator(s)
E0690611	**ADDEND: Quantitative and Molecular Analysis of 7,12-Dimethylbenz[a]- anthracene-induced Mutations in the Model Blue Rat: Comparison of Mutagenesis in the Trangene *lacI* with the Endogenous Gene** NCTR protocol E0690601 was undertaken in order to validate the use of the Big Blue rat as a model for determining the *in vivo* mutagenicity of potential human toxicants. Results from Exp. 1 uncovered unanticipated issues concerning the nature of mutagenic responses in the Big Blue model. These results suggest experiments not included in the original protocol that may resolve these issues. Necessitates using addn'l animals to complete exp. 1,2, and 3.	Manjanatha, Mugimane* Shelton, Sharon D.
E0694901	**The Effect of P53 Null Phenotype on Bleomycin-induced Stress Protein Elicitation *In Vivo* in Transgenic Mice** 1. Investigate the structure of the stress protein (sp) 70 and 90 genes by Southern blot in the 8-10 week old p53 null mouse in comparison with C57BL/6 control mouse. 2. Investigate the stress protein metabolic turnover (synthesis 35S-labeling) as a reflection of gene expression in the control homozygous C57BL/6 (+/+) and the null p53 homozygous TSG (-/-) mice as elicited by bleomycin (BL) at 1,2.3,4 and 5 mo. of age (during the G1-phase of the cell cycle) by polyacrylamide gel electrophoresis (PAGE), and their levels of radiolabeling calculated by computerized electronic area measurements. If stress proteins (sps) are absent in bone marrow nuclei of 1 month old p53 null mice (sp synthesis is dependent on the presence of the p53 gene) or if their expression is below the level of measurement then the protocol will be discontinued at test group 1, see below. 3. Investigate the phosphorylation patterns of sps as a reflection of gene expression as elicited by BL using the same animal types, time frames and techniques as in Objective 1. 4. To identify and examine nuclear polypeptides other than sps for synthesis and phosphorylation levels as possible biomarkers of metabolic alterations and gene expression during phases of the cell cycle in control and homozygous p53 null mice following administration of BL.	Pipkin, James L.* Hinson, William G. Lyn-Cook, Lascelles E. Manjanatha, Mugimane Shaddock, Joseph G.
E0694911	**ADDEND: The Effect of P53 Null Phenotype on Bleomycin-induced Stress Protein Elicitation *In Vivo* in Transgenic Mice** Requesting an additional 16 male pups in order to investigate the assessment of stress protein metabolism phenomenon in 2 wk. Old male pups.	Pipkin, James L.*
E0695801	**Mutant Frequencies and Types of Mutations Induced by Rat Carcinogens in the *Hprt* and *lacI* Genes of Big Blue Fisher 344 Rats** 1. To determine the mutant frequencies at the endogenous reporter gene *Hprt* in T-lymphocytes from the spleens of Fischer 344 rats following exposure to five mutagens: Aflatoxin B_1, N-hydroxy-2-acetylaminofluorene, benzo(a)pyrene, 2-amino-3-dimethylimidazo quinoline, and tris(1-aziridinyl)phosphine sulfide; 2. Determine the mutant frequencies at the endogenous gene *Hprt* and exogenous gene *lacI* from transgenic rats exposed to a mutagen selected from the five compounds examined in Objective 1; 3. Determine the types of mutations produced in the *Hprt* and *lacI* genes in the mutants induced in Objective 2.	Heflich, Robert H.* Aidoo, Anane Casciano, Daniel A. Manjanatha, Mugimane Mittelstaedt, R. A.
E0695811	**ADDEND: Mutant Frequencies and Types of Mutations Induced by Rat Carcinogens in the *Hprt* and *lacI* Genes of Big Blue Fisher 344 Rats** Addendum requesting treatment by gavage for 48 male Fisher 344 rats to be performed by Animal and Diet Prep contract personnel.	Heflich, Robert H.*

Project Number	Title/Objective	Principal*/ Co-Principal Investigator(s)
E0697501	**The Frequency and Types of Spontaneous Mutations Found in the *Hprt* and *lacI* Genes of Lymphocytes from Transgenic Big Blue Rats** 1. To determine the frequency of spontaneous mutation at the *Hprt* and *lacI* loci in pre-weanling, young (four-month-old) and old (18-month-old) Big Blue rats; 2. To determine the types of mutations present in the mutants from Objective 1.	Aidoo, Anane* Bishop, Michelle E. Heflich, Robert H. Lyn-Cook, Lascelles E. Mittelstaedt, R. A.
E0697511	**ADDEND: The Frequency and Types of Spontaneous Mutations Found in the *Hprt* and *lacI* Genes of Lymphocytes from Transgenic Big Blue Rats** The approved master project (E0697501) has not yet begun, and no animals have been allocated for it as yet. We have recently developed a method for expanding mutant rat lymphocyte clones from the approx. 100,000 cells per colony that are scored as mutants in our 96-well assay dishes to cultures containing several million cells. It is important to alter the experimental procedure of E06975.01 to take advantage of the new technology. Taking advantage of this technology, however, necessitates switching most of the animals used in the project from female to male.	Aidoo, Anane*
E0697701	**Evaluation of Chemical-Induced Mutagenesis in Transgenic Mice Containing the ϕX174 am3** Establishing the experimental parameters necessary to demonstrate a mutant frequency of 1.5- to 2-fold above background; Establishing the sensitivity of the am3 mouse model to mutagenic carcinogens and germ-cell mutagens expected to produce DNA damage at A:T base pairs. Where possible, compare the sensitivity of the ϕX174 system with that of other *in vivo* mutational systems; Establishing several basic properties of the ϕX174 am3 assay by determining the tissue or organ specificity of responses to certain carcinogens and by determining the patterns of mutations detected by the assay.	Valentine, Carrie R.* Burkhart, James G. Casciano, Daniel A. Heflich, Robert H. Malling, Heinrich
E0697711	**ADDEND: Evaluation of Chemical-Induced Mutagenesis in Transgenic Mice Containing the ϕX174 am3** Addendum requesting a minor addition of the protocol to bring a more conclusive and precise evaluation of the ϕX174 transgenic mouse system. An additional experiment will help us find out if DNA from non replicating cells may lead to the lower sensitivity in the ϕX174 assay. Requesting 3 pregnant ϕX174 transgenic mice.	Valentine, Carrie R.*
E0699101	**Influence of Dietary Restriction on Somatic Mutation and Antioxidant Enzymes Induced by Exposure of Female and Male Fischer 344 Rats to Bleomycin** To determine the frequency of occurence of lymphocytes bearing a mutant form of the *Hprt* gene as an indicator of DNA damage in caloric restricted and in *ad libitum* rats following exposure to bleomycin (BL); To determine how the activity of antioxidant enzymes such as catalase, glutathione peroxidase, and glutathione reductase relates to the mutant frequencies determined from the above objective; To determine the activity of the electron transport systems as an indicator of mitochondrial function during drug exposure; and To evaluate the integrity of mitochondrial DNA in BL treated rodents.	Feuers, Ritchie J.* Aidoo, Anane Desai, Varsha G.

Project Number	Title/Objective	Principal*/ Co-Principal Investigator(s)
E0700201	**The Development of Transgenic Mice Harboring Bacteriophage ϕX174 with Sites Specific for Detecting Mutations at Guanosine:Cytosine Nucleotides, Small Frameshifts, and Extended Deletions** To find specific mutations in bacteriophage ϕX174 that render the bacteriophage non-infectious and that will revert to plaque-forming ability only when mutation occurs by specific mechanisms: 1. Base substitution at a G:C base pair or 2. Frameshift caused by deletion of one or two nucleotides. An additional objective is to determine the feasibility of using ϕX174 to detect 3. The deletion of an extended sequence. Phage harboring these mutations will be used to construct a transgenic mouse model for measuring mutations *in vivo.*	Valentine, Carrie R.* Burkhart, James G. Fane, Bentley N. Goldman, Neil Grieshaber, Charles
E0700211	**ADDEND: R.O.W. Task Order #476 - Support for Hamster and Human Genome Mapping Project** Addendum requested to support modification to T.O. #476 where 320 additional hrs. are needed because data from the PI's collaborators at another institution has been delayed. Without this data, database cannot be created, nor can it be analyzed.	Valentine, Carrie R.*
E0701401	**The Use of Antioxidants in Single and in Mixture to Study the Effects of Dietary Vitamins on Genotoxicity Produced in Rats Treated with the Mammary Carcinogen** 1. To determine the genotoxic activity of dimethylbenz(a)anthracene (DMBA) and bleomycin (BL) by the cytokinesis-block micronucleus and *Hprt* assays in Fischer 344 rats that have been given a mixture of vitamin C, vitamin E and β-carotene and selenium by gavage; 2. To determine the mechanism underlying the inhibitory action of the dietary antioxidants by determining their effects on: a) spectra of induced mutations in *Hprt* gene in lymphocytes, b) oncogene (H-ras, K-ras) and tumor suppressor gene, p53 expression, c) programmed cell death (apoptosis), d) the activities of glutathione peroxidase, and glutathione S-transferase during DMBA and BL exposures.	Aidoo, Anane* Desai, Varsha G. Lyn-Cook, Lascelles E. Manjanatha, Mugimane McGarrity, Lynda J. Morris, Suzanne M.
E0701801	**Validation of the Mouse Targeted *Tk*+/- *In Vivo* System for Use in Mutagenicity Studies** 1. To expand a colony of transgenic *Tk*+/- mice using breeding of tk+/- founders and C57Bl/6 mice, and to transfer the *Tk*+/- genotype to a C57Bl/6 background; 2. To determine spontaneous mutant frequencies at the *Tk* and *Hprt* loci of splenic T-lymphocytes for mice of different ages; 3. To induce mutations in *Tk*+/- transgenic mice using treatment with the point mutagen ENU and the clastogens BLM and y-radiation, and to measure the kinetics of mutant induction at the *Tk* and *Hprt* loci; 4. To breed transgenic *Tk*+/- parents in an attempt to derive *Tk*-/- knockout mice, and study the biological signifcance of the *Tk* gene in mice; 5. To determine how the *Tk*-/- genotype may effect mutant frequencies at the *Hprt* locus.	Dobrovolsky, Vasily N.* Dass, Subbaraj B. Heflich, Robert H.
E0704101	**Measurement of H-*ras* Codon 61 CAA AAA Mutation in Mouse Liver DNAs using the MutEx/ACB-PCR Genotypic Selection** 1. Quantify somatic mutations in liver DNA of mice treated with 4-aminobiphenyl in order to establish and evaluate MutEx/ACB-PCR genotypic selection as an approach for human risk assessment; 2. Determine whether or not the MutEx/ACB-PCR genotypic selection is sensitive enough to measure the spontaneous frequencies of H-ras codon 61 CAA AAA mutation in three different mouse models: $B_6C_3F_1$, C57BL/6, and the Pms2 mismatch repair-deficient, transgenic mouse.	Parsons, Barbara L.* Heflich, Robert H.

Project Number	Title/Objective	Principal*/ Co-Principal Investigator(s)
E0704111	**ADDEND: Measurement of H-*ras* Codon 61 CAA AAA Mutation in Mouse Liver DNAs using the MutEx/ACB-PCR Genotypic Selection** Due to failure of a freezer, liver tissues being stored collected under the master protocol were thawed. The livers of the 1-month post-treatment time point of the newborn mouse assay were the ones destroyed. Additional animals and resources are being requested in order to repeat the one-month timepoint of the $B_6C_3F_1$ newborn mouse assay.	Parsons, Barbara L.*
E0704701	**Modulation of Gene Expression in Chemical Carcinogenesis: Analysis of Aflatoxin B_1 Induced Gene Expression in Human Hepatocytes** 1. Verify aflatoxin B_1 (AFB_1) effects on steady state mRNA levels of eight genes previously identified by differential hybridization of a gene filter array to be aflatoxin B_1 (AFB1)-responsive in human hepatocytes. Use Northern blot, RT-PCR and/or RNA protection assay to establish AFB_1 time and dose response curves for maximal gene expression and also determine the minimum dose at which gene expression can be detected. 2. Identify additional AFB_1-induced genes using differential display PCR (DD-PCR) and differential hybridization of a high density filter array utilizing mRNA from human hepatocytes treated with low, moderate and cytotoxic levels of AFB_1. Evaluate selected genes as described for objective #1. 3. Distinguish genes involved in toxicological response to AFB_1 exposure from those that contribute to the carcinogenic response by comparing the gene expression profile of human hepatocytes treated with the hepatotoxic noncarcinogenic chemical, acetaminophen. 4. Compare gene expression of selected genes in human hepatocytes treated with known rat liver chemical carcinogens, including 2-acetylaminofluorene.	Harris, Angela J.* Casciano, Daniel A.
E0705501	**Evaluation of the Genotoxicity of the Phytoestrogen, Coumestrol, in Human Lymphoblast Cells that Differ in the Mutational Status of the p53 Tumor Suppressor Gene** 1. To confirm the clastogenicity of coumestrol by the micronucleus assay; 2. To confirm the mutagenicity of coumestrol at the *HPRT* locus; 3. To determine if coumestrol induces large scale chromosomal damage such as that detected by the Tk mutation assay; 4. To determine if the toxicity of coumestrol is due to apoptosis; 5. To determine the effect of coumestrol on the cell-cycle.	Domon, Olen E.* Bishop, Michelle E. Chen, James J. McGarrity, Lynda J. Morris, Suzanne M.
E0706001	**The Effect of Amiloride on the Induced Mutation Frequency in Human Lymphoblastoid Cells** It is becoming increasingly clear that when the apoptosis pathways are blocked, either due to a mutation in an apoptosis regulatory gene or to the effects of an exogenous agent, cells with DNA damage may survive and proliferate. The proposed studies are designed to determine: 1. If amiloride suppresses apoptosis in the AHH-1 Tk+/ cell line; 2. If amiloride affects the cell cycle, primarily at the G2/M transition; 3. If amiloride increases either the spontaneous or topo-II-inhibitor-induced mutant frequency at both the *Hprt* and Tk locus.	McGarrity, Lynda J.* Chen, James J. Domon, Olen E. Morris, Suzanne M.

Project Number	Title/Objective	Principal*/ Co-Principal Investigator(s)
E0706301	**The Development of a Genotypic Selection Assay and Analysis of the Age-Specific Patterns of Mutant** 1. To develop a genotypic selection assay (GSA) allowing a direct measurement of mutant frequencies and molecular analysis of mutation in any non-polymorphic endogenous sequence and in any tissue; 2. To determine the spontaneous mutant frequencies (MFs) and age-associated accumulation rats (Ars) in highly (Exon 3) and poorly (Exon 4) mutable regions of *Hprt* coding sequence in the *Hprt* lymphocyte mutation assay; 4. To compare the *in vivo* persistence of elevated MFs in *Hprt* exons 3 and 4 induced after exposure to ENU.	Khaidakov, Magomed* Aidoo, Anane
P00389	**Demonstrate and Train Visiting Scientists in *In Situ* Perfusion, Isolation, and Primary Culture of Rat Hepatocytes** To demonstrate and train visiting scientists in the procedures utilized in our laboratory associated with the *in situ* perfusion, isolation, and primary culture of rat hepatocytes.	Shaddock, Joseph G.*
P00397	**Enrichment of *ras* Gene Sequence Through Hybrid Selection** The livers of these animals will be used to isolate genomic DNA and this DNA will be used to develop a hybridization method for the gene-specific enrichment of the *ras* oncogene. The outcome of this experiment will determine how many animals will be required in a planned protocol on the uses of genotypic selection methods to measure chemically-induced *ras* mutations.	Parsons, Barbara L.*

PROJECTS COMPLETED FY1999

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0693301	**Tumor Prone P53-Deficient Transgenic Mice (TSG-p53TM): A Potential System to Augment the Sensitivity of Carcinogenicity Testing and for Studying the Mutational Basis of Tumors** The genome instability of p53-deficient mice will be determined by monitoring the frequency of spontaneous mutations in the *Hprt* biomarker gene of T-lymphocytes from the spleen. The time for appearance of tumors in the p53 heterozygotes will be compared with that for the wild type mice; *ras* and p53 mutations will be examined in such tumors. The frequency of mutations that arise on exposure of these animals to the carcinogens benzo[a]pyrene and dimethylnitrosamine in a neonatal carcinogenicity protocol will be monitored at the *Hprt* locus in T-lymphocytes. The spectrum of carcinogen-induced mutations in the *Hprt* locus will be determined by PCR and DNA sequencing; this information may indicate mutational mechanisms, serve as a fingerprint of environmental exposure, and permit risk assessment.	Casciano, Daniel A. * Harris, Angela J. Heflich, Robert H. Manjanatha, Mugimane
E0693311	**ADDEND: Tumor Prone P53-Deficient Transgenic Mice (TSG-p53TM): A Potential System to Augment the Sensitivity of Carcinogenicity Testing and for Studying the Mutational Basis of Tumors** Addendum submitted to perform an additional series of experiments - requesting additional animals to be treated with ENU.	Dass, Subbaraj B. *
E0693321	**ADDEND: Tumor Prone P53-Deficient Transgenic Mice (TSG-p53TM): A potential System to Augement the Sensitivity of Carcinogenicity Testing and for Studying the Mutational Basis of Tumors** Requesting additional dams to be purchase off-site due to poor littering and survival characteristics of the newborn transgenic animals. No change to number of neonatal, p53-transgenic pups to be treated, or in their manner of treatment.	Dass, Subbaraj B. *
E0693331	**ADDEND: Tumor Prone P53-Deficient Transgenic Mice: A Potential System to Augment the Sensitivity of Carcinogenicity Testing and for Studying the Mutational Basis of Tumors** Additional animals overshipped by outside supplier - addendum requesting to use additional animals and pups and add third chemical to study.	Dass, Subbaraj B. *
E0693501	**Development of Methods for the Biochemical Selection of Mutations** Establish biochemical selection methods to detect and quantify rare mutations in the DNA of mutagen-treated animals. The value of the *E. coli* mismatch binding protein (Muts), used with the polymerase chain reaction (PCR), as a biochemical selection for mutations in the *ras* oncogene will be evaluated.	Parsons, Barbara L. * Heflich, Robert H.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0695001	**Molecular Analysis of *In Vitro* Mutations in the Transgenic Rat2 Cells Exposed to DMBA and Tamoxifen: Comparison of Mutagenesis in the Transgene *lacI* with the Endogenous Gene *Hprt*** To determine the mutant frequency and mutation spectrum of the *lacI* transgene in Rat2 cells following exposure to DMBA and tamoxifen prior to evaluation in Blue Rat. To determine the mutant frequency and mutations spectrum of the endogenous *Hprt* reporter gene in Rat2 cells following exposure to DMBA and tamoxifen. Compare *in vitro* mutant frequencies and mutational spectra with those determined in the Big Blue rats *in vivo* from the Expt. 6906.	Manjanatha, Mugimane * Casciano, Daniel A. Harris, Angela J. Shaddock, Joseph G.
E0699601	**Evaluation of the Genotoxic Potential of Genistein in Human Lymphoblastoid Cells** To confirm the potential mutagenicity of genistein utilizing the TK/*HPRT* mutation assay; to determine if apoptosis can account for the toxicity of genistein; to characterize the effect of genistein exposure on the traverse of the cell-cycle; to evaluate the role of the p53 tumor suppressor gene in the response to genistein exposure by performing the experiments which address objectives 1,2, and 3 in both the AHH-1 *tk* (p53) and L3 (*tk*;p53) human lymphoblastoid cell lines.	Morris, Suzanne M. * Chen, James J. Domon, Olen E. McGarrity, Lynda J.

CALORIC RESTRICTION GROUP

INTRODUCTION

It has been demonstrated that 40% caloric restriction (CR) alters many physiological processes in model rodent systems. The CR group has developed many physiological, biochemical, and morphological biomarkers to evaluate the response to CR in these test systems. Two issues of interest to NCTR and FDA form the basis for this group's activities at present. These are: 1) developing methodologies to implement these findings relative to product testing and evaluation; and 2) determining the relevance of these findings to human risk assessment.

FY1999 ACCOMPLISHMENTS AND FY2000 PLANS

A research protocol was developed in collaboration with the Center for Food Safety and Applied Nutrition (CFSAN) to resolve certain problems encountered in animal studies. That is, the increased weight and obesity of the rodent strains used in bioassays led to high mortality rates. Poor survival was noted in animals fed purified diets, such as the AIN-76, that use casein as their protein source. It was also necessary to examine the use of dietary restriction as a means of increasing survival and reducing individual variation. This study was designed to develop new methodologies and a comprehensive database to support future animal studies.

The physiological testing phase of this experiment began in FY98 after the development of a data acquisition and process control system to support these studies. It was demonstrated that the NCTR CD rat (a Sprague Dawley Caesarian-derived strain) has the best survival potential of any Sprague Dawley strain tested to date and that this strain is an excellent rodent model for future toxicological studies. Importantly, the mortality rate for the CD rat was reduced to 12.5%, 12.5%, and 5% when the total caloric content of the diet was reduced by 10%, 25%, and 40%, respectively. These data suggest that small changes in diet can increase longevity. These survival data will be important in developing protocols for animal diets in future drug toxicity studies.

A second project on caloric restriction attempts to determine if the physiological, metabolic, and molecular changes that occur with caloric restriction in rodents are similar in humans. Members of the CR Group are actively collaborating with the staff of the Department of Surgery, University of Tennessee at Memphis to measure similar biological endpoints in patients that have undergone a surgical procedure (tummy tuck) that produces caloric restriction. The most notable finding is that the activity of complex III of the electron transport system was found to be significantly elevated in obese women compared to thin women. When this result was examined in light of a kinetic evaluation, it suggested that electron transport operates more efficiently in lymphocytes from thin women compared to obese women.

FY2000 GOALS

1. Determine the ideal level of caloric intake and develop methods for implementation of animal studies.

2. Complete the evaluation of a human model system to determine the effect of reduced dietary intake on physiological, biochemical, metabolic, and molecular endpoints.

PUBLIC HEALTH SIGNIFICANCE

The collaborative study between NCTR and CFSAN is designed to resolve problems associated with the chronic bioassay and risk assessment. Data from this study indicate that a reduction in calorie intake results in increased survival rates, reduced individual variability, and reduced spontaneous tumor incidence. Further, maintenance of animals on a purified diet resulted in similar survival rates in the NCTR CD rat. These data will enable the FDA to more accurately establish guidelines for bioassays. The "Memphis" study provides an opportunity to compare biomarkers in an animal and human model of caloric restriction. These data will be important in establishing the efficacy of caloric restriction in humans and its effect on human health. These data will also provide the FDA with an evaluation of the reliability of extrapolation of rodent data to the human population in any number of situations important to the FDA.

ACTIVE PROJECTS FY1999

Project Number	Title/Objective	Principal*/ Co-Principal Investigator(s)
E0692401	**Effect of Different Levels of Caloric Restriction (CR) on Physiological, Metabolic, Biochemical, Immunological, Molecular, and Body Composition** 1. To determine how various levels and durations of CR affect physiological function, enzymes related to intermediary and drug metabolism, hormonal regulation, blood chemistry, etc; 2. Determine the relationship between body fat (BF), fat free mass (FFM), total body water (TBW), and total body electrical conductivity (TOBEC) as a function of strain, age, mass, and nutritional status in rats; 3. Validate and automate the use of a new noninvasive electromagnetic scanning device to measure BF, FFM, and TBW and to compare the results to a conventional chemical fat extraction technique; 4. Determine if CR alters the relative quantity and disposition of various types of lipids such as cholesterol, phospholipids, free fatty acid, etc. in various tissues, as well as in urine, feces, and blood serum; 5. Develop control data related to CR that can be used by CFSAN to evaluate the toxicity and efficacy of low calorie foods, food additives, and food substitutes; 6. Determine temporal and environmental factors that modulate the effects of CR; 7. Develop experimental methods for utilizing a low level of CR to increase the survival rate and to decrease variablity in the chronic bioassay; to provide the concomitant control data for comparison; 8. Develop control data for a reference purified diet that has been formulated to conform to a long-term nutrient requirements of rodent animal models typically utilized in toxicology and nutrition studies.	Duffy, Peter H.* Leakey, Julian E. Allaben, William T. Allen, Laura J. Chanderbhan, Ronald F. Feuers, Ritchie J. Hart, Ronald W. Hass, Bruce S. Leakey, Julian E. Lewis, Sherry M. Lyn-Cook, Beverly A. Pipkin, James L. Turturro, Angelo
E0692411	**ADDEND: Effect of Different Levels of Caloric Restriction on Physiological, Metabolic, Biochemical, Immunological, Molecular and Body Composition Variables in Rats** Requesting 40 additional male Sprague-Dawley rats be added to this protocol to serve as ad libitum controls in a dietary restriction (DR) study.	Duffy, Peter H.*
E0692421	**ADDEND: Evaluation of Cellular Responses in Rats -- Cell Proliferation Study by Flow Cytometric Cell Cycle Analysis** Flow cytometric cell cycle analysis for cell proliferation studies was missing in the original master protocol of the CFSAN-NCTR collaborative study E06924.01. Therefore, this addendum proposes to efficiently utilize the available tissues from E0692401 to perform flow cytometric cell cycle analysis to obtain or accumulate data on cell cycle effects by dietary restriction. Will utilize bone marrow, kidney, spleen, and thymus tissues that were not previously designed for study in E0692401 for further evaluation using flow cytometric cell cycle analysis to study cell proliferation activities of tissues obtained from rats that received various levels of dietary restriction; will be conducted without the need of requesting addn'l animals.	Lu, MingHsiung* Tang, Ning

REPRODUCTIVE TOXICOLOGY LABORATORY

INTRODUCTION

One of the most important challenges facing the FDA is the identification and regulation of chemicals, food additives, and biological therapies that may induce developmental toxicity. Congenital malformations affect 7% of the population at birth, another 7% have low birth weights, and at least 25% of pregnancies end in spontaneous abortion. Further, over 20% of all infant deaths are due to birth defects and funding for children with developmental disabilities represents a major portion of federal and state budgets for health services.

Dr. Dan Sheehan, Dr. Robert Blair and Dr. Hong Fang discuss a computer model of data collected for the Endocrine Disruptors Knowledge Base.

Over one dozen chemicals, the majority of which are FDA-regulated, are recognized as human teratogens; many more agents are suspected human teratogens. However, no chemical regulated by the FDA has been tested for developmental toxicity in pregnant women which puts a heavy burden on laboratory animal research.

FY1999 ACCOMPLISHMENTS AND FY2000 PLANS

NCTR is recognized as an international leader in defining the normal and estrogen-altered reproductive tract developmental profile in the rat. The level of expertise, combined with a well-defined estrogen database created over the past 20 years, led to the initiation of a project to create and validate a computerized knowledge base to aid in the regulatory decision process. This project, funded by a series of grants from the FDA's Office of Women's Health (OWH), has led to a series of papers on the Quantitative Structure Activity Relationships (QSAR) models for chemical binding to the estrogen receptor. QSAR models are being developed for estrogen binding to rodent alpha-fetoprotein and human testosterone-estradiol-binding globulin. Over 200 chemicals have been evaluated in an estrogen receptor assay, an activity partially funded by a Cooperative Research and Development Agreement (CRADA) with the Chemical Manufacturers Association (CMA). This program was reviewed in 1999 by the Endocrine Disrupters site visit team of the NCTR Science Advisory Board and found to have made excellent progress since its last review.

Other projects within the Reproductive Toxicology Laboratory have led to an increased understanding of the role of developmental toxicants on the induction of birth defects including neural tube defects. Expression of 5,10-methylenetetrahydrofolate reductase (MTHFR) was decreased in mouse embryos using antisense oligonucleotide methodology. Neural tube defects were found in embryos with decreased activity of

MTHFR. Co-injection of 5-methylenetetrahydrofolate with the MTHFR antisense oligonucleotide decreased the incidence of embryos with neural tube defects. When this technology was applied to the investigation of the role of the folate receptor in neural tube closure, neural tube defects were produced when embryos were injected with an antisense oligonucleotide for the folate receptor. Co-injection of 5-methyltetrahydrofolate with the antisense for the receptor decreased the incidence of neural tube defects. We have also examined expression of the receptor in organogenesis-stage mouse embryos. The receptor mRNA is expressed in nearly all cells in the GD8 embryo, and is expressed in many different cell types in the GD9 embyro. These findings suggest that MTHFR and the receptor may play roles in normal closure of the neural tube and that decreases in activity of these proteins may be involved in neural tube defects.

FY2000 GOALS

1. Develop improved methods and new strategies for detecting and predicting the developmental toxicity in laboratory animals and the human population, focusing on reproductive tract development, whole embryo development, and the molecular biology of development.

2. Develop a knowledge base for the binding of chemicals that bind to the estrogen and androgen receptor.

PUBLIC HEALTH SIGNIFICANCE

Exposure to FDA-regulated estrogens and anti-estrogens occurs in tens of millions of women. Oral contraceptive exposure occurs in over 100,000 pregnancies each year. Approximately five percent of U.S. women will receive tamoxifen during their lifetime. Phytoestrogen exposure of the human population via food is virtually universal; infants consuming soy formula are exposed to the highest doses. Estrogenic activity is found in environmental chemicals, such as plastics and pesticides, and in FDA-regulated products. Thus, it is important to understand the varying toxicological and pharmacological properties of these compounds as well as their common mechanism of action. In order to provide the FDA with computational expertise in this area, the Laboratory has constructed an endocrine disrupter knowledge base with the capability of predicting hormonal activity of untested chemicals. These models will continue to be applied to specific regulatory issues across a number of FDA product centers. The endocrine disrupter knowledge base will help to identify additional areas in which further research will aid in the making of regulatory decisions.

ACTIVE PROJECTS FY1999

Project Number	Title/Objective	Principal*/ Co-Principal Investigator(s)
E0689001	**Effects of Maternal Diabetes and Insulin on Fetal Expression of Insulin-like Growth Factor and Insulin-like Growth Factor Binding Protein mRNAs** To determine whether experimentally inducting diabetes in pregnant rats by treatment with streptozotocin will alter fetal expression of insulin-like growth factor (IGF) mRNAs and insulin-like growth factor binding protein (IGFBP) mRNAs. To determine to what extent restoring normoglycemia in pregnant diabetic rats by treatment with insulin will restore the normal pattern of fetal expression of insulin-like growth factor mRNAs and insulin-like growth factor binding protein mRNAs.	Streck, Randal D.* Fishman, Renata B. Contrea, Joseph Seamon, Ken
E0689011	**Effects of Diabetes and Insulin on Fetal Expression of Insulin-like Growth Factor (IGF) and Insulin-like Growth Factor Binding Protein (IGFBP) mRNAs** To quantitate the level of IGF and IGFBP mRNA expression in the livers of fetuses carried by diabetic dams and insulin-treated diabetic dams compared to normal control dams. This will enable us to refine and define quantitatively the results we are obtaining from the experiments an *in situ* analyses described in protocol E0689001.	Rajaratnam, Veeraramani S.* Streck, Randal D. Webb, Peggy
E0692901	**Toxicant Effects on Neural Cell Adhesion Molecule and N-cadherin during Mouse Neural Tube Closure** To determine the optimum time of neural cell adhesion molecule (NCAM) and N-cadherin expression in the closing CD-1 mouse neural tube; to quantitate changes in neural fold NCAM and N-cadherin levels following embryonic exposure to valproic acid, lithium or heat *in vivo*.	Hansen, Deborah K.*
E0696001	**Further Studies on the Mechanism of Valproic Acid (VPA)-Induced Embryotoxicity** 1. Determine a sensitive period for VPA-induced neural tube defects (NTDs) in rat embryos treated *in vitro*; 2. Determine if VPA produces hypomethylation of DNA in treated rat embryos *in vitro*; 3. Determine S-adenosylmethionine/S-adenosylhomocysteine (SAM/SAHC) ratios in control and VPA-treated embryos during the sensitive period; 4. Determine if VPA produces hypomethylation of DNA in embryos treated with the drug *in vivo*; 5. Determine if inactivation of methionine synthase increases the embryotoxicity of VPA.	Dial, Stacey L.* Grafton, Thomas F. Terry, Ketti K.
E0697301	**Mechanism of Tamoxifen Developmental Toxicity and Neoplasia: Tamoxifen Effects on the Rat Uterine Insulin Like Growth Factor System** 1. To define the ontogeny of insulin-like growth factor (IGF) system mRNA expression in the developing rat uterus; 2. To determine the uterine cell types in which IGF system mRNAs are expressed; 3. To determine the effects of diethylstilbestrol (DES), tamoxifen (TAM) and ICI 182,780 on IGF system mRNA expression at selected developmental stages.	Branham, William S.* Sheehan, Daniel M. Collins, Jerry

Project Number	Title/Objective	Principal*/ Co-Principal Investigator(s)
E0697601	**Dose-Response of Retinoic Acid (RA)-induced Stress Protein (SP) Synthesis and its Correlation with Developmental Toxicity in CD-1 Mice** Determine the incidence of limb malformations on gestation day 17 (GD 17) and the extent of synthesis of SPs in limb bud tissue determined 2.5 hr after RA treatment following various doses of RA administered on GD 11. Determine the incidence of cleft palate on gestation day 17 (GD 17) and the extent of synthesis of SPs in craniofacial tissue determined 2.5 hr after RA treatment following various doses of RA administered on GD 13.	Hansen, Deborah K.* Hinson, William G. Lyn-Cook, Lascelles E. Pipkin, James L. Shaddock, Joseph G. Goering, Peter Stratmeyer, Melvin
E0697611	**ADDEND: Dose-Response of Retinoic Acid-Induced Stress Protein Synthesis and Its Correlation with Developmental Toxicity in CD-1 Mice: T.O. #490 (Ident. and Analysis of GEL Image Blots)** Addendum submitted to cover ADP Task Order #490 requesting ADP resources.	Laborde, James B.*
E0698501	**The Role of Reactive Intermediates in Carbamazepine (CBZ)-Induced Embryotoxicity** To determine if the anti-oxidant, glutathione (GSH), is able to decrease CBZ-induced embryotoxicity in mouse embryos; To determine if inhibition of GSH synthesis by L-buthionine-(S,R)-sulfoximine (BSO) increases the embryotoxicity of CBZ; To determine if the antioxidative enzyme, superoxide dismutase (SOD), decreases CBZ-induced embryotoxicity; To determine if the prostaglandin H synthase inhibitor, aspirin, decreases CBZ-induced embryotoxicty; To determine if treatment with 12-o-tetradecanoylphorbol-13-acetate (TPA) which activates the release of arachidonic acid increases CBZ-induced embryotoxicity; To determine if treatment with eicosatetraynoic acid (ETYA), an inhibitor of both prostaglandin H synthase and lipoxygenase decreases CBZ-induced embryo-toxicity.	Dial, Stacey L.* Grafton, Thomas F.
E0698511	**ADDEND: The Role of Reactive Intermediates in Carbamazepine (CBZ)-Induced Embryotoxicity** Requesting additional animals due to inconsistencies generated in animal data requiring a repeat of experiment.	Hansen, Deborah K.*
E0699811	**ADDEND: Task Order #483 & #493 - LIMS Implementation and Review of Heart Rate Variation Analysis Software** Addendum requested to add ADP resources needed for Task Order #483 - Memphis Study: LIMS Implementation.	Duffy, Peter H.*
E0700801	**Bioassay of Reproductive Tract Toxicities Caused by Genistein and Methoxychlor in Sprague-Dawley Rats** To perform a battery of analyses which will determine the capacity of two xenoestrogens, genistein and methoxychlor, administered via two exposure routes to induce estrogen responses and reproductive tract developmental toxicities in rodents. This objective covers the ovary and uterus. A continous feeding protocol will be compared to a 5-day injection protocol at 4 periods of development.	Branham, William S.* Hass, Bruce S.

Project Number	Title/Objective	Principal*/ Co-Principal Investigator(s)
E0700811	**ADDEND: Bioassay of Reproductive Tract Toxicities Caused by Genistein and Methoxychlor in Sprague-Dawley Rats** It has been determined that support for the feeding portion of E0700801 should include the use of the InLife System for maintaining a record of body weights and feed consumption. The first group of rats to be entered into the InLife System will be delivered to the study on 12/17/97.	Sheehan, Daniel M.* Branham, William S.
E0702001	**Antisense Knockouts of Genes in the Folate Pathway and Effects on Neural Tube Development** 1. To determine if knocking out 5,10-methyltetrahydrofolate reductase (MTHFR) activity in mouse embryos *in vitro* produces neural tube defects; 2. To determine if addition of exogenous 5-methyltetrahydrofolate is able to overcome the lack of MTHFR activity and produce closed neural tubes in mouse embryos treated *in vitro*; 3. To determine if addition of exogenous methionine is able to overcome the lack of MTHFR activity and produce closed neural tubes in mouse embryos treated *in vitro*; 4. To determine if knocking out methionine synthase (MS) activity in mouse embryos *in vitro* produces neural tube defects; 5. To determine if addition of exogenous methionine is able to overcome the lack of MS activity and produce closed neural tubes in mouse embryos treated *in vitro*; 6. To determine if exogenous vitamin B12 is able to overcome the lack of MS activity and produced closed neural tubes in mouse embryos treated *in vitro*; 7. To determine if knocking out methionine adenosyltransferase (MAT) activity in mouse embryos *in vitro* produces neural tube defects; 8. To determine if addition of exogenous methionine is able to overcome the lack of MAT activity and produce closed neural tubes in mouse embryos treated *in vitro*; 9. To determine if addition of exogenous 5-methyltetrahydrofolate is able to overcome the lack of MAT activity and produce closed neural tubes in mouse embryos treated *in vitro*.	Hansen, Deborah K.* Grafton, Thomas F. Streck, Randal D.
E0703401	**Indices of Biotin Nutrition** To determine the human requirement for biotin in normal individuals and in individuals in certain circumstances in which biotin status may be impaired. Specific Aim#4 (which will be accomplished at NCTR) will determine whether biotin of similar severity to that observed in human pregnancy can cause significantly increased rates of fetal malformation in the mouse. In the pilot mouse study, marginal biotin deficiency in mouse dams that caused an increase in 3-HIA excretion similar to that seen in human pregnancy produced 10% incidence of cleft palate in the fetal mouse.	Hansen, Deborah K.* Laborde, James B.
E0703501	**Predictability of Animal Data for Human Developmental Toxicity** 1. Retrieve reports of human data from published literature and FDA files for therapeutic agents for which there are adequate data to indicate either positive effects or no effect; 2. Retrieve reproductive and developmental toxicity study data in laboratory animals from FDA files or directly from pharmaceutical companies on the same products; 3. Extract specific data elements into a database for qualitative and quantitative comparison; 4. Evaluate data using the expertise of pharmacology/toxicology and clinical/epidemiology project participants; 5. Conduct statistical analyses, initially using multiple regression analyses and correlation approaches, with more sophisticated analyses as the data permit; 6. Draw conclusions about the predictability of animal testing data, and recommend design improvements as appropriate.	Hansen, Deborah K.* Chen, James J. Fisher, Edward Fitzsimmons, Jack Gaylor, David W. Kimmel, Carole Laborde, James B. O'Conner, Anita M. Vega, Amarylis

Project Number	Title/Objective	Principal*/ Co-Principal Investigator(s)
E0703601	**An Investigation of the Possible Developmental Toxicity of St. John's Wort (Hypericin)** 1. To determine if administration of St. John's Wort by gavage alters embryonic growth and development in rats; 2. To determine if maternal administration of St. John's Wort to rats prenatally and during the first three weeks after birth alters early postnatal growth and survival; 3. To determine if maternal administration of St. John's Wort to rats prenatally and during the first three weeks after birth alters learning and various behaviors later in life.	Hansen, Deborah K.* Chen, James J. Ferguson, Sherry A. Laborde, James B. Wilkes, Jon G.
P00358	**Training in the Estrogen Developmental Toxicity Bioassay** This training protocol is the first phase of a project funded by the Office of Women's Health, FDA. In order to carry out the study, Pathology Associates, Inc. (PAI) technicians need to be trained in animal sacrifice, tissue removal and processing, instrumentation, morphometric techniques, aspects of project management and procedures for data collection, recording, retrieval, reduction and summarization.	Sheehan, Daniel M.* Branham, William S. Burroughs, Cynthia D. Medlock, Kevin L.
P00385	**Validation of the Rat Estrogen Receptor Competitive Binding (RERCB) Assay** To validate the RERCB assay in our laboratory as a basis for providing later data for the Estrogen Knowlege Base.	Hass, Bruce S.* Branham, William S. Sheehan, Daniel M.
P00388	**Identification of Molecular Markers of Peroxisome Proliferator-Activated Receptor-Gamma Activation in the Rat Fetus** 1. To determine which rat fetal tissues express peroxisome proliferator-activated receptor (PPARY); 2. To identify, from a set of genes regulated by PPARY in adults, genes that are coexpressed in the same fetal tissues as express PPARY.	Streck, Randal D.*

PROJECTS COMPLETED FY1999

Project Number	Title/Objective	Principal*/ Co-Principal Investigator(s)
E0686721	**ADDEND: Additional Investigations on the Mechanism of Valproic Acid (VPA)-Induced Embryotoxicity** 1. Determine if administration of pantothenic acid is able to decrease the incidence of VPA-induced neural tube defects in mice treated *in vivo*; 2. If pantothenic acid does decrease the incidence of VPA-induced neural tube defects, to determine if it does so by altering the amount of VPA which is transferred to the embryo.	Hansen, Deborah K. * Grafton, Thomas F.
E0689101	**Alterations in Reproductive Tract Morphology and Biochemistry in Rats Treated Neonatally with Phytoestrogens** 1. To determine if phytoestrogens, when given neonatally, alter estrogen receptor and progesterone receptor concentrations in the uterus and brain at 6 and 10 months in the same manner as DES. 2. To determine if phytoestrogens, when given neonatally cause the same morphological alterations in the female reproductive tract at 6 and 10 months as DES. 3. To determine if phytoestrogens, when given neonatally, elicit the same induction of the c-*ras*, c-*myc* and c-*fos* oncogenes as DES.	Burroughs, Cynthia D. * Faber, Ken Hughes, Claude Whitten, Patricia Pohland, Albert
E0689111	**ADDEND: Alterations in Reproductive Tract Morphology and Biochemistry in Rats Treated Neonatally with Phytoestrogens Treated Neonatally with Phytoestrogens** Requesting additional support from Pathology contractor (PAI) and Computer contractor (R.O.W.) in the development of computer assisted 3-dimensional analysis of structural alterations in the reproductive tracts of rats.	Burroughs, Cynthia D. * Sheehan, Daniel M. Pohland, Albert
E0696901	**Enzymatic Oxidation of 17β-Estradiol: Role of the Products in Hormone Action** Estradiol metabolites formed by peroxidase or tyrosinase interact with the estrogen receptor.	Medlock, Kevin L. * Sheehan, Daniel M. Pohland, Albert
P00370	**Development of an Estrogen Knowledge Base for Research and Regulation** The purpose of this effort is to identify active elements in estrogen and estrogenic compounds, using the data in the NCTR estrogen database and commercial analysis and modeling tools. The application of traditional and advanced QSAR techniques to this ideal data set should either conform the existence of active moieties or identify confounding factors that point the way towards further research. In either case, the result of this effort will be an estrogen database with a predictive capability, called a knowledge base.	Sheehan, Daniel M. * Gaylor, David W. Harrison, Nancy E. Lay, Jackson O. Perkins, Roger G. Shvets, Vadim F. Strelitz, Richard A. Ulmer, William C.

FY1999 PUBLICATIONS*

1. Aidoo, A., Desai, V.G., Lyn-Cook, L.E., Chen, J.J., Feuers, R.J. and Casciano, D.A. Attenuation of bleomycin-induced *Hprt* mutant frequency in female and male rats by calorie restriction. Mutation Research, Accepted: 9/24/99. **(E0699101)**

2. Casciano, D.A., Aidoo, A., Chen, T., Mittelstaedt, R.A., Manjanatha, M. and Heflich, R.H. *Hprt* Mutant Frequency and Molecular Analysis of *Hprt* Mutations in Rats Treated with Mutagenic Carcinogens, Mutation Research Special Issue, Accepted: 8/12/99. **(E0695801)**

3. Chen, J.B., Dobrovolsky, V.N. and Heflich, R.H. Development of a Mouse Cell Line Containing the Phix174 Am3 Allele as a Target for Detecting Mutation. Mutation Research, Accepted: 5/20/99. **(E0697711)**

4. Dass, S.B., Bucci, T.J., Heflich, R.H. and Casciano, D.A. Evaluation of the transgenic p53+/- mouse for detecting genotoxic liver carcinogens in a short-term bioassay. Cancer Letters, 143:81-85, 1999. Accepted: 5/7/99. **(E0693301)**

5. Delongchamp, R.R., Chen, J.B., Heflich, R.H. and Malling, H. An Estimator of the Mutant Frequency in Assays using Transgenic Animals, Mutation Research, 440:101-108, 1999. Accepted: 1/6/99. **(Collaborating with Biometry)**

6. Dobrovolsky, V.N. Efficient human IFN-gamma expression in the mammary gland of transgenic mice. Journal of Interferon and Cytokine Research, 19:137-144, 1999. Accepted: 10/10/98. **(NA)**

7. Dobrovolsky, V.N., Casciano, D.A. and Heflich, R.H. *Tk*+/- mouse model for detecting *in vivo* mutation in an endogenous, autosomal gene, Proc. National Academy of Science, 423:125-136, 1999. Accepted: 11/2/98. **(E0701801)**

8. Dobrovolsky, V.N., Chen, T. and Heflich, R.H. Molecular analysis of *in vivo* mutations induced by N-ethyl-N-nitrosourea in the autosomal Tk and X-linked *Hprt* genes of mouse lymphocytes. Environmental & Molecular Mutagenesis, 34:30-38, 1999. Accepted: 6/5/99. **(E0701801)**

9. Feuers, RJ. The effects of dietary restriction on mitochondrial dysfunction in aging, Annals of the New York Academy of Sciences, 854:192-201, 1998. Accepted: 10/1/98. **(NA)**

10. Hansen, D.K., Young, J.F., Laborde, J.B., Wall, K.S. and Holson, B. Pharmacokinetic Considerations of Dexamethasone-Induced Developmental Toxicity in Rats. J. Toxicological Sciences, 48:230-239, 1999. Accepted: 5/21/99. **(E0663812)**

11. Kulkarni, S.G., Casciano, D.A., Harris, A.J. and Mehendale, H. Differential protooncogene expression in Sprague Dawley and Fischer 344 rats during 1,2-dichlorobenzene-induced hepatocellular regeneration, Toxicology, Accepted: 9/13/99. **(E0704701)**

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* **(_ _ _ _ _ _ _ _)** ***Indicates the related NCTR project number; NA-Not Applicable***

12. Laborde, J.B., Wall, K.S., Bolon, B.N., Kumpe, T.S., Patton, R.E., Zheng, Q., Kodell, R.L. and Young, J.F. Haematology and serum chemistry parameters of the pregnant rat. Laboratory Animals, 33:275-287, 1999. Accepted: 2/8/99. **(E0695711)**

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VETERINARY SERVICES

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Histopathology processing of tissue specimens, taking it all apart to help NCTR researchers put it all together

Introduction

The Division of Veterinary Services (DVS) provides professional and technical support to the various NCTR research divisions in their efforts to conduct peer-reviewed scientific research that supports and anticipates the FDA's current and future regulatory needs. The Division provides administration for the Center's Animal Care and Use Program which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International). Included within the division are the contracted services for animal care, diet preparation, and pathology, all of which are staffed by on-site contract employees.

FY1999 Accomplishments

Immediate Office

During 1999, an additional veterinary medical officer certified by the American College of Laboratory Animal Medicine (ACLAM) was recruited and hired. The Division provided oversight and veterinary management of all laboratory animals and housing facilities at NCTR. Division personnel completed and submitted annual reports assuring compliance with Federal regulations and National Institute of Health (NIH) guidelines relative to the Animal Care and Use Program. Personnel participated in semi-annual program reviews, facility inspections, and experimental protocol reviews as part of the NCTR Institutional Animal Care and Use Committee proceedings. The director serves as a member of the FDA Research Animal Committee and its AAALAC International accreditation subcommittee which performs "mock" AAALAC site visits of the various FDA centers prior to actual site visits by AAALAC representatives. Division personnel serve as government project officers for the pathology services, animal care and diet preparation, and rodent bedding contracts. During 1999, contracts for pathology services and rodent bedding were successfully re-competed. The Request for Proposals (RFPs) was prepared and issued for the re-competition of the animal care and diet preparation contract that will take place in mid-FY2000. The Division is responsible for breeding, rearing, and/or acquiring all experimental animals used on-site. A new rodent surgical suite was established and used to perform rodent ovariectomies in support of multigeneration toxicology studies. DVS veterinarians

assisted the NCTR Safety Staff in coordinating preparations for a planned protest of NCTR's use of nonhuman primates by an animal rights group. Such preparations included giving interviews to the local press. The Division's personnel also served as instructors in the on-site technician certification program, which was authorized by the American Association for Laboratory Animal Science (AALAS). DVS co-authored one scientific publication in 1999.

Animal Care/Diet Preparation Services

During 1999, eight contract animal care personnel attained the highest level (Laboratory Animal Technologist) of certificate by the American Association for Laboratory Animal Science (AALAS). The average number of experiments supported per month by contract animal care personnel was 73. These experiments entailed as a minimum, the daily animal care support of an average of 5,953 rodents, 16 rabbits, and 121 rhesus monkeys. Technical manipulations for these studies included one or more of the following procedures: tattooing (7,492 animals), vaginal lavages (16,110), tumor palpations (10,814), injections (23,000 SQ, IM or IV), oral gavage (12,850 rodents and nonhuman primates), behavioral testing (27,821), and blood collection (3,258). Contract diet preparation personnel provided consultation and nutritional support and diet preparation for several carcinogenicity studies including fumonisin B_1, chloral hydrate, malachite green and leucomalachite green, and urethane, funded through the Interagency Agreement with the National Institute for Environmental Health Sciences' National Toxicology Program. Personnel also gave a presentation on dietary energy utilization and protein calorie malnutrition to the NRC Sub-Committee on nonhuman primate nutrition and submitted two manuscripts for publication in the scientific literature. During 1999, diet preparation personnel produced 63,200 lbs. of dosed diet, autoclaved 103,030 lbs. of rodent diet, 5,500 liters of dosed water, and 2,294 lbs. of sized dietary pellets. Quality assurance personnel performed 3,320 quality control audits of contractor-performed procedures.

Pathology and Pathology-Related Services

During 1999, six trainees completed the Laboratory Technician apprenticeship training program and have become eligible to take the histotechnician registry exam in the late Fall. Personnel worked to develop the use of the ACCESS to replace Paradox to review, organize and summarize pathology data. Data slides were prepared for pathology personnel and other NCTR researchers using Powerpoint and a Polaroid ProPalette 7000 Film Recorder with developing being accomplished with a Jobo Autolab Automatic 35mm processor. Contract personnel also adapted special procedures such as the following in support of NCTR research projects:

- Preparation of tissues for the evaluation of sexually dimorphic nuclei in the brain of rats. Included development of a procedure to produce a flat surface; digital imaging of brains, and outlining specific nuclei and other anatomical reference points of the brains to facilitate two- dimensional and three-dimensional analysis of the sexually dimorphic nuclei.

- Developed embryo culture techniques (*in vitro*) to examine early organogenesis in single embryos. The embryos were injected with antisense oligodeoxyribonucleotides into the amniotic sacs of embryos to determine if decreased expression of the targeted genes has adverse effects on development.

- *In situ* hybridization procedures.

- Sphingoid base-level analysis for fumonisin B_1

- Image analysis and 3-D reconstruction for genistein, vindozolin, nonylphenol and ethinyl estradiol on the sexually dimorphic nucleus of the preoptic area of the rat brain.

- Image analysis and two-dimensional data for methoxychlor on the sexually dimorphic nucleus of the preoptic area of the rat brain.

- Used the MCID M5+ software for image analysis and three-dimensional reconstruction.

- Elisa assays for neurotoxicology.

Contract employees also accomplished the following methods development for the endocrine disruptor studies:

- Immunohistochemical detection of estrogen receptor alpha in rat uterus and mammary gland fixed in Bouin's solution or paraformaldehyde.

- Immunohistochemical detection of estrogen receptor beta in rat ovary fixed in formalin.

- Immunohistochemical detection of androgen receptor in rat testes, epididymis, and prostate fixed in Bouin's solution or formalin.

- Trained animal care technicians in proper methods for vaginal lavage of rodents to assess estrous cycles.

- Trained pathology technicians in methods of preparation and interpretation of vaginal cytology specimens.

- Immunohistochemical methods to selectively distinguish both spermatogonia and sertoli cells, making them easily quantified by automated image analysis. These methods should significantly improve the efficiency of collection accuracy of the data.

- Dual staining with immunohistochemical and Periodic Acid Schiffs-Hematoxylin (PAS-H) allows identification and quantitation of immunoreactive germ cell lineages in specific stages of the epithelium of the seminiferous tubules.

- Sertoli cell nuclei negatively stain with Proliferating Cell Nuclear Antigen (PCNA) antibody. All the nuclei of the germ cell lineages along the basement membrane of the seminiferous tubule stain positively by Immunohistochemistry.

- Antibody specific for Protein Gene Product 9.5 (PGP-9.5) selectively stains spermatogonia making them easily quantified by automated image analysis.

The contractor developed an experienced nonhuman primate necroposy team to provide necropsy coverage for any unexpected deaths in the non-human primate colony. Personnel supported the peer reviews for the chloral hydrate and fumonisin B_1 studies at NCTR; provided pathology consultation services to CDER in the review of the NDI for Pantrapazole; purchased, set up, and implemented the Hamilton-Thorne Integrated Visual Optical System (IVOS) to analyze rat sperm samples for motility; and determined the cause for early mortality in *thymidine kinase* knockout mice (glomerulosclerosis possibly due to autoimmune disease).

During 1999, the contract employees authored or co-authored 11 publications or presentations and were awarded the Society of Toxicology Board of Publications Award for the Best Paper in Toxicology and Applied Pharmacology for 1998.

FY2000 PLANS

- Continue to support the research mission of NCTR while seeking ways to become more efficient in doing so.

- Successfully re-compete the animal care/diet preparation contract to obtain the best services available at a reasonable cost.

- Support, where possible, the concept of a cooperative agreement with a local community college for the training of technicians (both animal care and pathology) to relieve service contractors of the burden of on-site training of technicians at the government's expense.

- Continue to supply methods development where needed to support the NIEHS IAG work at NCTR.

- Continue a quality laboratory animal care program that is consistent with State and Federal laws, regulations, and guidelines.

PUBLIC HEALTH SIGNIFICANCE

FDA's mission, pure and simple, is to protect and promote the nation's public health. Animal related studies, as those being conducted by the NCTR research community, greatly enhance the Agency's ability to meet this public health mission. The Division of Veterinary Services has the facilities, equipment and personnel to support this vital interdisciplinary research.

The "gold standard" for laboratory animal care and use programs is accreditation by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). Such accreditation is widely accepted by the scientific community and indicates that the accredited organization conforms with all government policies and regulations and that it endorses the highest quality care for the animals involved in their animal use activities. DVS personnel, working through the FDA Research Animal Council (FRAC), have assisted, and will continue to assist, FDA Centers in obtaining and maintaining accreditation of their animal care and use programs by AAALAC International.

Active Projects FY1999

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0666502	**Pigskin - Dermal Acute Study** To determine the No Observable Adverse Effects Level (NOAEL) of topical applications of LP1846 Liquid Gun Propellant on male Miniature Hanford Pigs using hematological and serological assays.	Witt, William M.*
E0666503	**LP1846 Liquid Gun Propellant Dermal Toxicity** To determine the effects of removal of LP1846 Liquid Gun Propellant by rinsing after specific post-exposure time intervals on male Miniature Hanford Pigs using hematological assays.	Witt, William M.* Gosnell, Paul Parker, Robert M. Talbot, A
E0666504	**Liquid Gun Propellant Dermal Toxicity Study** 1. Use a topical exposure encompassing approximately 10% skin surface area in male miniature swine to ascertain possible detectable differences between direct cutaneous exposure and exposure by fabric patch on the development of adverse clinical effects; 2. Characterize, by sequential skin biopsies, the progression of skin lesions resulting from direct cutaneous application of LP1846.	Witt, William M.* Gosnell, Paul Parker, Robert M. Talbot, A

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7. Schnellman, J.G., Pumford, N.R., Kusewitt, D.F., Bucci, T.J. and Hinson, J.A. Deferoxamine delays the development of the hepatoxicity of acetominophen in mice. Toxicology Letters, 106:79-88, 1999. Accepted: 1/29/99. **(Collaborating with Neurotoxicology) (NA)**

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* **(_ _ _ _ _ _ _ _)** ***Indicates the related NCTR project number; NA-Not Applicable***

9. Turturro, A., Witt, W.M., Lewis, S.M., Hass, B.S., Lipman, R. and Hart, R.W. Growth curves and survival characteristics of the animals used in the biomarkers of aging program. Journal of Gerontology, Accepted: 6/28/99. **(Collaborating with Ofc. of Dir./Imm. Ofc.) (E0050400)**

10. Von Tungeln, L.S., Xia, Q., Bucci, T.J., Heflich, R.H., and Fu, P.P. Tumorigenicity and liver tumor ras-protooncogene mutations in CD-1 mice treated neonatally with 1- and 3-nitrobenzo[a]pyrene and their trans-7,8-dihydrodiol and aminobenzo[a]pyrene metabolites. Cancers Letters. In press, Accepted: 10/30/98. **(Collaborating with Biochem. Tox.) (E0687901)**

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MOLECULAR EPIDEMIOLOGY

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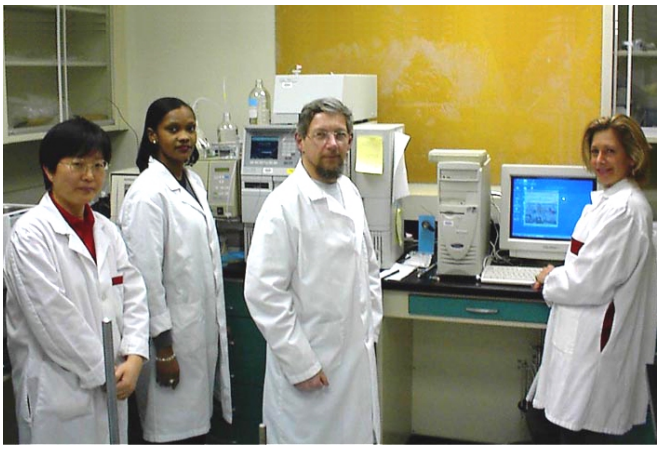

The Division combines human exposure biomonitoring efforts to studies of individual genetic susceptibility.

INTRODUCTION

The strategic goals of the Division are: 1) the identification of genetic polymorphisms that influence drug and carcinogen metabolism, individual cancer susceptibility, and therapeutic drug efficacy; 2) the conduct of epidemiological studies for post-market surveillance of chemical toxicants found in foods, drugs, cosmetics, and medical devices; 3) human exposure biomonitoring and DNA adduct detection; 4) the extrapolation of the results of animal bioassays and of mechanistic studies to humans; and 5) the development and validation of "DNA Microarray Technology" for human diagnostics.

The intent is to better understand the mechanisms of human carcinogenesis; to provide an estimation of human exposure to direct and indirect-acting carcinogens; to assess the importance of inter-individual differences in carcinogen and drug bioactivation, detoxification, or induced changes in gene expression; and to suggest intervention strategies for human cancer prevention. Accordingly, research has provided new knowledge on the identification of subpopulations that are not only more susceptible to chemical carcinogens, but also those that are likely to experience adverse drug reactions or decreased therapeutic drug efficacy. Research has been focused on the foodborne heterocyclic amines, aromatic amines, and polycyclic aromatic hydrocarbons, and on widely used drugs including selected benzodiazepines, antihistamines, drugs inducing peroxisomal proliferation or oxidative stress, estrogens, anti-estrogens and endocrine disruptors, as well as on tobacco usage. Projects on the etiology of human cancers of the colon/rectum, pancreas, larynx, breast, ovary, prostate, lung, urinary bladder, bone marrow, and esophagus are ongoing.

Studies to identify genetic polymorphisms that influence drug and carcinogen metabolism, individual cancer susceptibility, and therapeutic drug efficacy:

- Metabolic polymorphisms, DNA repair, and individual cancer susceptibility.
- Genetic and epigenetic regulation of cytochrome P450 1A2.
- Polymorphisms of cytochrome P450 1B1 and tissue-dependent expression.
- Polymorphisms of cytochromes P450 2A6 and P450 2E1.

- Polymorphisms of phenol and estrogen sulfotransferases.
- Polymorphisms of glutathione S-transferases A1, A2, and P1.
- Inter-individual variation in DNA repair capacity.
- Substrate specificity and activity of COX-1 and COX-2 toward metabolic activation of foodborne carcinogens.
- Gender-specific variation in drug metabolism.

1. Chemoprevention.

   a) Modulation of expression of multi-drug resistance genes by micronutrients and dietary factors.

   b) Flavnoids' and isoflavonoids' effects on cytochrome P450s, GSTs and NATs.

   c) Modulation of expression of proteases (kallikrein, maspin, NES1) in pancreatic cancer cells by flavonoids.

   d) Flavonoids and isoflavonoids: biochemical and therapeutic application in pancreatic cancer.

   e) DNA methylation, DNA methyltransferase, and homocysteine toxicity.

Epidemiology and post-market surveillance for chemical toxicants found in foods, drugs, cosmetics, and medical devices:

2. Etiology of human cancers.
   a) Etiology of human colorectal cancer: role of dietary heterocyclic amines.

   b) Etiology of human breast and prostate cancers in African-Americans.

   c) Etiology of human pancreatic cancer: role of carcinogen and drug exposures, chronic pancreatitis, and dietary imbalance.

Human exposure biomonitoring and DNA adduct detection:

Biomarkers of exposure and susceptibility for breast, prostate, ovarian, laryngeal, esophageal, lung, colon, and urinary bladder cancers.

Extrapolation of the results of animal bioassays and of mechanistic studies to humans:

Evaluation of the neonatal mouse bioassay as an alternative bioassay for selected benzodiazepines, antihistamines, chloral hydrate, drugs inducing peroxisomal proliferation or oxidative stress, synthetic and natural estrogens, and endocrine disruptors, including chlorinated hydrocarbon pesticides and dinitroaniline herbicides.

International efforts in molecular epidemiology and biotechnology:

Organization of the Molecular Epidemiology Group of the American Association for Cancer Research.

3. Development and validation of "DNA Microarray Technology" for assessing individual risk for cancer susceptibility and recurrence, adverse drug reactions, and therapeutic drug efficacy.

Experiment No.: E03000.01

Title: **Methods for Discovering and Scoring Single Nucleoside Polymorphisms (Risk-Tox Chip Program)**

Principal Investigator: Fred F. Kadlubar

Agency Benefit: The development and validation of "DNA Microarray Technology" for human diagnostics, with emphasis on the identification of susceptible subpopulations.

Specific Objectives: NCTR partners with Genometrix® through a CRADA, to develop a "Risk-Tox" DNA microarray platform for rapid, high throughput genotyping. The goal is to be able to genotype patients for all the major enzyme variants that would enable us to predict carcinogen susceptibility, adverse drug reactions, chemotherapeutic drug efficacy and individualized dosing. Such efforts could have a major impact on the ability to understand the likelihood of adverse health effects in susceptible subpopulations. The initial emphasis will be the conduct of ongoing validation studies, comparing standard methodologies of genotyping to automated, large-scale genotyping on a robotic workstation and the planning of large-scale genotyping for ongoing epidemiologic studies. This will involve collaboration with the University of Arizona Cancer Center on the recurrence of colo-rectal polyps in a cohort of some 6,000 patients, who are part of a clinical intervention trial involving wheat bran fiber, ursodeoxycholic acid, and selenium+ celecoxib supplementation. If this effort is successful, it will then be extended to a larger sample set from the Centre de Medecine Preventive (Nancy, France), which involves about 20,000 individuals with complete medical histories available from 1955 to the present.

Accomplishments: Project is under review.

Expected Agency Impact: The immediate scientific impact of the research plan is two-fold. First, the development of a rapid screening methodology for genetic polymorphisms in risk-related alleles is expected to greatly impact the rate at which data can be collected and analyzed in population-based, risk-assessment studies. Currently, screening large sample sizes for a single allelic variant by polymerase chain reaction/restriction fragment length polymorphisms (PCR/RFLP) analysis requires several weeks and a large laboratory staff. Second, the development of rapid, automated screening methodologies (*e.g.*, analysis of several hundred alleles/1000 persons/day) to identify individuals genetically at risk for adverse health effects would greatly facilitate FDA review of individual drug disposition studies and FDA post-market

surveillance, based upon “profiles of individual risk” for agents with known toxicities in a given genetic background. The technology transfer to industry could have a revolutionizing effect on diagnostic medicine by allowing physicians to prescribe drug dosage more accurately and on an individual basis.

Experiment No.: E06946.01
Title: A Case Control Study of Pancreatic Cancer and Aromatic Amines
Principal Investigator: Fred F. Kadlubar

Agency Benefit: To establish a basis for Agency recommendations concerning diet and individual susceptibility to pancreatic cancer.

Specific Objectives: To determine of critical factors in pancreatic cancer etiology and factors affecting individual cancer susceptibility by: 1) examination of phenotypic changes in major enzymes of carcinogen metabolism (NATs,CYPs, GSTs) during progression to disease states; 2) examination of genetic polymorphisms in these enzymes to identify genetic risk factors for the disease; and 3) examination of combination of phenotypic and genetic variation in combination with carcinogen exposure to identify factors that determine individual risk to the disease.

Accomplishments: Analysis of molecular epidemiological data from NCTR’s completed, case-control study on pancreatic cancer has begun and initial data indicate that the slow *NAT1*4* allele is a significant risk factor. New laboratory studies on chronic pancreatitis, which is the strongest predisposing factor for the development of pancreatic cancer, has shown a five-to-5 fold increase in the levels of *CYP1A1*/2, *CYP1B1*, and *CYP3A4*, with the latter present at the highest levels, comparable to about 5-10% of that found in human liver. Variation in expression of *GST* phenotype has been assessed, with *GSTA2*, known to be critical for carcinogen detoxification and protection from lipid peroxidation, being the major isoform in normal pancreas, was found to be strongly down-regulated in pancreatitis. A novel *GST* polymorphism in *GSTA2* was also discovered.
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Expected Agency Impact: This project is expected to provide an assessment of the relative roles of dietary and environmental carcinogens in human pancreatic cancer and to result in appropriate recommendations for protecting public health.

Experiment No.: E-6947.01
Title: Role of Acetylation and *N*-Oxidation in Colo-rectal Cancer
Principal Investigator: Fred F. Kadlubar

Agency Benefit: To establish a basis for Agency recommendations concerning diet and individual susceptibility to colo-rectal cancer.

Specific Objectives: To determine critical factors in colo-rectal cancer etiology and factors affecting individual cancer susceptibility by: 1) examination of phenotypic changes in major enzymes of carcinogen metabolism (NATs,CYPs, GSTs) during

progression to disease states; 2) examination of genetic polymorphisms in these enzymes to identify genetic risk factors for the disease; 3) examination of combination of phenotypic and genetic variation and hormonal effects in combination with carcinogen exposure to identify factors that determine individual risk to the disease; and 4) examination of epigenetic status of the estrogen receptor in normal, distal and tumor colon tissues.

Accomplishments: Analysis of molecular epidemiological data from the completed, case-control study on colo-rectal cancer at UAMS has begun and dietary questionnaires are being evaluated that will give exposure estimates to foodborne heterocyclic amines. Recent laboratory studies have defined new polymorphisms in sulfotransferases and led to a series of publications characterizing these enzyme systems. Similar studies on UGTs and a *N*-hydroxy amine reductase have also been carried out and are reported.

In future studies, NCTR will be collaborating with investigators at the Arizona Cancer Center to evaluate the role of heterocyclic amines and tobacco smoke carcinogens in polyp recurrence, and the possible modulating effects of *NAT1, NAT2*, and *CYP1A2* polymorphisms on risk associated with dietary consumption of heterocyclic and aromatic amines. The associations between exposures, susceptibility, and *ras* gene mutations also will be assessed. The effects of dietary selenium (Se) supplementation, unsaturated fat and cruciferous vegetable intake, polymorphisms in *GST P1* and *GST T1*, and the efficacy of a COX-2 inhibitor (Celecoxib) on polyp recurrence and progression to colo-rectal cancer also will be assessed in a large cohort of >4000 patients, using our DNA chip (E03000.01). The central hypotheses involve the role of COX-2 expression in colon tumorigenesis and its ability to utilize fatty acid hydroperoxides as co-substrates. These hydroperoxides, which may be formed as a consequence of lipid peroxidation due to high fat diets or low antioxidant status, can be detoxified by the Se-dependent glutathione peroxidases and by the GSTM1 and GST T1, both of which exhibit genetic polymorphisms. Thus, dietary Se supplementation and high activity GST alleles may be protective against colo-rectal cancer, especially in combination with Celecoxib intervention.

DNA hypermethylation studies also have been recently undertaken to determine if abnormal site-specific DNA methylation occurred in the estrogen receptor promoter region in human colon cancer since the loss of estrogen receptor expression has been associated with hormone resistance in some cancers. Using the methylation-specific PCR (MSP) method, 88% of human colon tumors contained hypermethylated sites, while 100% of adjacent and distal normal tissues contained hypomethylated sites in same region. In the same tissues that hypermethylation was observed, 75% of the tumors showed a decrease in COX-1 expression and a 70% increase in COX-2 expression.

Expected Agency Impact: This project is expected to provide an assessment of the relative roles of dietary and environmental carcinogens in human colo-rectal cancer and has already resulted in dietary recommendations for protecting public health (American Cancer Society, American Institute for Cancer Research). In addition, these studies

offer a rationale for recent epidemiological data suggesting that women on hormone replacement therapy and women who have taken oral contraceptives have a lower risk of colon cancer.

Experiment No: E6952.01
Title: Effect of Dietary Restriction on Carcinogenesis in Rats Fed Methyl-Deficient Diets.
Principal Investigators: Ming Chou and Lionel Poirier

Agency Benefit: Supports product review by developing more accurate tests for toxicity based upon mechanism of action and recognizes the role played by dietary deficiency in enhancing susceptibility to drug toxicity.

Specific Objectives: To show the interactive effects of aflatoxin treatment, dietary methyl deficiency, and caloric restriction on liver carcinogenesis. The expected impact is two-fold: 1) to show the beneficial effects of caloric restriction in preventing cancer; and 2) to show that carcinogen exposure and micronutrient deficiency act synergistically to enhance tumor formation. Demonstration of the former would result in strengthening PHS recommendations to reduce cancer risk by cutting back on calories. Demonstration of the latter would cause further heed to be paid to folate deficiency as an enhancer of drug toxicity in humans.

Accomplishments: The clear demonstration that dietary restriction markedly inhibits the formation of neoplasia and of preneoplastic lesions in the livers of rats given a methyl-deficient diet with or without an initiating dose of aflatoxin B_1. These results further extend prior findings on the strong synergism in carcinogenesis between mycotoxins and dietary deficiencies of methyl donors.

Expected Agency Impact: Increased awareness within the Agency that the marginal folate deficiency commonly present in humans is a risk factor for increased susceptibility to the potential toxicity of a number of regulated products.

Experiment. No.: E66962.01
Title: *CYP1A2* Gene Methylation and Regulation in Human Liver
Principal Investigators: George Hammons and Beverly Lyn-Cook

Agency Benefit: Human cytochrome P450 IA2 (*CYP1A2*) is involved in the metabolic disposition of a large number of commonly used therapeutic drugs and is responsible for the metabolic activation of numerous promutagens and procarcinogens. Large inter-individual differences exist in the expression of this enzyme and understanding the fundamental mechanisms for inter-individual variation is of major importance in predicting therapeutic drug efficacy and individual cancer susceptibility.

Specific Objectives: Because of delays in tissue procurement, epigenetic studies to elucidate the mechanism of gene regulation for *CYP1A2* were not initiated until FY99. The Division's approach has been to correlate the methylation status of CpG sites within

or near identified regulatory elements in the 5'-flanking region of *CYP1A2* with its level of expression in human liver samples, in particular the methylation status of the CCGG site located adjacent to an AP-1 site, using restriction enzymes and PCR analysis.

Accomplishments: Individual samples were found to vary in the methylation status at this site with hypermethylation being associated with reduced expression of *CYP1A2.* Since differences in the methylation status of *CYP1A2* were detected in these samples, studies were also conducted to assess whether or not differences existed in the levels of the enzyme, DNA methyltransferase, that is known to affect methylation status. Levels were found to vary among the samples and were significantly higher in samples from cigarette smokers. Further analysis of other CpG sites in potentially important regulatory elements of the *CYP1A2* gene will be critical. For these continued studies, methylation-specific PCR will be employed to determine methylation status.

Expected Agency Impact: Understanding the mechanistic basis for the inter-individual differences in an enzyme involved in drug metabolism and carcinogen activation will provide an important contribution to FDA's mission to regulate products and protect public health.

Experiment No: E06978.01
Title: Chemical Carcinogenesis: Epithelial Cells in Breast Milk
Principal Investigator: Christine Ambrosone

Agency Benefit: Post-market surveillance of chemical toxicants found in foods, drugs, cosmetics, and medical devices for their potential to cause DNA damage in the breast of lactating women.

Specific Objectives: To identify potential breast carcinogens by characterization of DNA adducts in exfoliated ductal epithelial cells in human breast milk, and to identify the mutagenic activity of the milk itself. To evaluate associations between questionnaire data and biologic markers of exposure/effect and further identify genotypes that may result in higher levels of adducts and mutagenicity.

Accomplishments: The study population of 50 breastfeeding women was examined, exfoliated cells enriched for epithelial cells, and DNA was extracted and evaluated for adducts using ^{32}P-postlabelling-TLC and -HPLC. Thus far, adducts were found in 66% of specimens, and 38% of samples were mutagenic. Adduct levels correlated with mutagenicity, but not with urinary cotinine, a marker of exposure to tobacco smoke. Initial data using ^{32}P-postlabelling-TLC indicate the presence of 4-aminobiphenyl (ABP)-DNA adducts in these cells and this was correlated with the presence of the rapid (*NAT2*) acetylator genotype. An additional study population of 100 women has been recruited, and samples obtained, in order to corroborate these findings by more selective mutagen-extraction methods and by ^{32}P-postlabelling-HPLC (with a synthetic standard). Moreover, a method has been developed for recovery of *N*-hydroxy-ABP from breast milk that should allow its identification by HPLC/accelerator mass spectrometry.

An invited symposium lecture was presented at the annual AACR meeting, an additional paper is in preparation, and further studies are in progress.

Expected Agency Impact: This study will provide information about the source of chemical contaminants in human breast milk and mechanisms of their toxicity, including assessment of risk for breast cancer.

Experiment No: E6981.01
Title: Short-term Dietary Supplementation in Manipulation of DNA Methylation and Methyl Metabolism in Mice.
Principal Investigator: Lionel Poirier

Agency Benefit: Supports product review by developing more accurate tests for predicting toxicity based upon mechanism of action.

Specific Objectives: To determine whether the mechanisms linking cancer causation with dietary methyl insufficiency are applicable to other pathologies induced by toxic agents. Increasing the parallels between cancer and other pathological effects would result in enhanced understanding of mechanisms and capability for risk assessment.

Accomplishments: Last year's achievements on this project consist principally in: 1) extending the evidence that abnormal methylation plays a causative role in an increasing number of pathologies, in addition to cancer; and 2) providing data that the abnormal functioning of the enzyme DNA methyltransferase contributes to the carcinogenic process.

Expected Agency Impact: An improved predictability of risk for those agents appearing to exert their carcinogenic and other toxic effects via abnormal methyl group metabolism.

Experiment No: E6990.01
Title: Role of Human CYP1B1 Drug Metabolism and Carcinogenesis
Principal Investigator: Fred F. Kadlubar (replaces G. Tang)

Agency Benefit: Human cytochrome P450 1B1 (*CYP1B1*) is involved in both testosterone and estrogen metabolism and in carcinogen and drug biotransformation in extrahepatic tissues. The large inter-individual variation in the expression of this enzyme and a better understanding of its function in these tissues are of major importance in predicting therapeutic drug efficacy and individual cancer susceptibility.

Specific Objectives: To use well-characterized anti-peptide antibodies that the Division has developed for immunohistochemical studies on tissue localization in humans and to validate these results by *in situ* localization. An addendum to this protocol seeks to investigate a novel finding: the intense localization of *CYP1B1* mRNA and protein in the neurons of the brain with specific localization in the nuclear membrane (other tissues

show cytoplasmic distribution). The study also will investigate existing formalin-fixed paraffin blocks of primate brain to assess the potential role of *CYP1B1* in neuronal function. Finally, it will study normal and tumor-derived brain cell lines that recently have been found to contain *CYP1B1* in order to assess whether or not its expression is under hormonal control.

Accomplishments: *CYP1B1* was identified as a major CYP in human lung, breast, prostate, brain and ovary; and a polymorphic variant ($Leu^{432}Val$) of the *CYP1B1* Val variant was shown to be a high activity allele. In addition, population studies and a pilot case-control study indicated that the high activity allele was a risk factor for prostate cancer. The Division also will apply this approach to other hormonally related cancers such as breast and ovary, as well as lung cancer, multiple myeloma, and myelodysplastic syndrome in collaboration with ongoing studies at Wake Forest University and at the M.D. Anderson Cancer Center.

Expected Agency Impact: This effort will provide fundamental knowledge on the therapeutic or potentially harmful effects in *CYP1B1* variants of hormonal therapies or oral contraceptive usage, as well as those related to possible gender-determined effect in the brain.

Experiment No.: E7015.01

Title: Breast Cancer in African-American Women: Metabolic Modification of Dietary and Hormonal Factors

Principal Investigator: Christine Ambrosone

Agency Benefit: The conduct of epidemiological studies for post-market surveillance of chemical toxicants found in foods, drugs, cosmetics, and medical devices in relation to human breast cancer.

Specific Objectives: To conduct a case-control study of breast cancer, with questionnaire data on known and suspected breast cancer risk factors. To evaluate risk associated with those exposures, particularly in relation to inter-individual variability in metabolism of dietary and other carcinogens and endogenous and exogenous steroid hormones.

Accomplishments: Establishment of infrastructure for molecular epidemiologic study (questionnaire development, protocols and equipment for blood processing and specimen banking, recruiter and interviewer hiring and training, development of data bases for participant tracking and questionnaire data, etc.).

- Enrollment, to date, of 270 cases and 65 controls into study – response rates far superior to those in earlier case-control study in the same locales.
- Adaptation of Food Frequency Questionnaire to the African-American population in Arkansas.
- Biologic specimen bank established with DNA, serum, plasma and red blood cells from cases and controls.

- Grant for expanded study submitted to NCI 2/99 based on these pilot data, 'Breast Cancer in the Lower Mississippi Delta'. Investigators: Ambrosone, Fontham, Erwin, et al.

Expected Agency Impact: To better understand the mechanisms of human carcinogenesis and to provide new knowledge on the identification of susceptible subpopulations [funded by FDA/Office of Women's Health (OWH), Public Health Service (PHS)/OWH, Department of Defense (DoD)].

Chemotherapy and radiotherapy are often used as adjuvant treatment following surgery for breast cancer, and many women undergo high dose chemotherapy, with or without bone marrow rescue. For many women, however, therapy does not prevent recurrence. In addition to the nature of the tumor itself, inter-individual variability in metabolism of chemotherapeutic agents, and in response to free radicals created by radiotherapy, may predict who will respond to treatment and who will not. The Division is completing a molecular epidemiologic study to determine the role of variability in metabolic phenotype and genotype of several glutathione *S*-transferases (GSTs) in response to treatment for breast cancer. Genetic polymorphisms may impact dose or type of agent given, and could have significant impact on therapeutic decisions. In specimens from more than 200 women with breast cancer, the study is evaluating laboratory data in relation to treatment and response, with consideration of other factors including patient and tumor characteristics. The results of this study could serve to improve the efficacy of chemotherapeutic drugs either by identifying subpopulations with improved outcomes or by providing the basis for individualized drug dosing.

Experiment No: 7017.01

Title: The Effects of Nicotine and Other Cigarette Components on Normal and Neoplastic Human Pancreatic Cells: the Role of Low Zinc Levels on Ras, Mdr-1 Genes Activation and Metabolizing Enzyme Activities as a Possible Risk Factor for Pancreatic Cancer

Principal Investigator: Beverly Lyn-Cook

Agency Benefit: The development of better biological assays to predict human genetic damage in specific genes involved in pancreatic toxicity.

The human pancreas is an organ highly susceptible to toxicity of various drugs regulated by FDA such as sulfonamides, thiazides, furosemide, estrogens, nicotine, and a variety of the AIDS agents. A number of these drugs cause pancreatitis, which is widely regarded as one of the strongest risk factors associated with the etiology of pancreatic cancer. Low levels of zinc have been associated with induction of pancreatitis. The *ras* genes are the most common genetic alterations found in human cancers; and understanding and evaluating the effect of *ras* oncogenes on other target genes, such as the multidrug resistance gene (*mdr-1*), could stimulate novel approaches for the development of new cancer drugs for pancreatic cancer. Examining various levels of zinc on nicotine-induced effects can assess the possible role of

micronutrients on regulation of *ras* expression. Pancreatic cancer has no established chemotherapeutic treatment to date.

Specific Objectives: To develop an *in vitro* human cell system using human pancreatic acinar cells to examine the effects of nicotine, its metabolites, and various chemoprotective agents. To examine the effects of zinc on activation of *ras* and *mdr-1*. To study the effects of these agents on the expression or induction of the *CYP1A1* and *CYP1A2*. To carry out RNA fingerprinting to determine the effects of these agents on other regulated genes. To determine if nicotine, a highly addictive drug, is also genotoxic to human pancreas and whether the micronutrient, zinc, could modulate these effects. To examine inter-individual differences in the expression of *Ki-ras* , *mdr-1* and other related genes in human pancreatic tissues grouped according to sex, age, race, and smoking status.

Accomplishments: Initial studies indicate that nicotine causes a mutation in codon *Ki-ras* 61 in normal pancreatic acinar cells (HP-8); however, mutational analysis is ongoing on other established cell lines to confirm this mutation. Reverse transcriptase-polymerase chain reaction (RT-PCR) revealed that nicotine-treated cells increased the expression of *Ki-ras* and increased the expression of *mdr-1* gene. Zinc down-regulated *Ki-ras* expression in nicotine-treated cells. RNA fingerprinting revealed that nicotine up-regulated NF-kb, *MnSOD, Kallikrein,* and *COX-2* expression and resulted in down-regulation of the *NES-1* gene. These genes were further examined in pancreatic cancer cell lines and found to be highly expressed. Further studies indicated that various chemopreventive agents could down-regulate these genes in the pancreatic cancer cells. Future studies are planned to further examine these genes and their possible role in pancreatic carcinogenesis.

Expected Agency Impact: This project will aid FDA in understanding mechanisms involved in pancreatic toxicity by different classes of drugs that are currently being regulated. These intermediate biomarkers could serve as surrogate endpoints for epidemiological studies by evaluating differences in metabolism by target organs such as the pancreas. Moreover, this *in vitro* assay is expected to aid in predicting the efficacy of chemopreventive agents in whole animals.

Experiment No.: E7021.01
Title: Prostate Cancer: Exposure, Susceptibility and DNA Adducts
Principal Investigator: Christine Ambrosone

Agency Benefit: The conduct of epidemiological studies for post-market surveillance of chemical toxicants found in foods, drugs, cosmetics, and medical devices in relation to human prostate cancer with emphasis on ethnic/racial differences in susceptibility.

Specific Objectives: To conduct a case-control study of prostate cancer, evaluating genetic and environmental risk factors for the disease. To characterize carcinogen-DNA adducts in human prostate tissue, and to evaluate adduct levels in relation to exposures and genetic polymorphisms. To determine whether or not there is a

difference between prostate cancer patients and control participants in the distribution of polymorphic alleles of genes involved in androgen metabolism. To determine whether or not there are racial/ethnic differences in adduct levels and in the distribution in any of these polymorphisms.

Accomplishments: To date, 130 men with prostate cancer and 40 healthy controls have been interviewed. Due to the use of prostate cancer survivors as recruiters, participation rates are excellent. DNA adduct analyses have also been conducted using tissue from radical prostatectomies, but these resources have been limited. However, initial data using ^{32}P-postlabelling-TLC indicated the presence of 2-amino-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-DNA adducts in these tissues and the adduct levels were correlated with the presence of the rapid (*NAT2*) acetylator genotype. Additional samples are being obtained in order to corroborate these by ^{32}P-postlabelling-HPLC (with a synthetic standard). Moreover, a method has recently been developed that will allow for combined adduct analyses and genotyping from prostate needle biopsies which is expected to greatly facilitate data collection.

Expected Agency Impact: To better understand the mechanisms of human carcinogenesis and to provide new knowledge on the identification of susceptible subpopulations (Funded by National Institute on Aging).

Experiment No.: E7043.01
Title: *In Vivo* Modeling of Steroid-Mediated Gender Effects in Drug Metabolism
Principal Investigators: Gail McClure and Fred F. Kadlubar

Agency Benefit: In 1993, the FDA lifted a ban on the participation of women of child-bearing age in early drug trials. With increased efforts to advance the understanding of sex differences and gender-specific issues in drug metabolism, CDER and OWH are now encouraging the participation of females in early clinical trials and incorporation of oral contraceptive (OC) or hormone replacement therapy (HRT) usage, menstrual cycle, and menopausal status into study design. The information gained from this study of steroid-mediated, gender effects is expected to aid in understanding the mechanisms of individual variability in drug-metabolizing enzymes associated with hormonal variations in women and should provide a basis for postmarket evaluations of therapeutic compounds and adverse drug reactions.

Specific Objectives: To characterize the activity of *CYP1A2*, *CYP2D6* and *CYP3A4* in female and male subjects with regard to age, race, phase of the menstrual cycle, pregnancy, oral contraceptive usage, menopause, and HRT. To measure estradiol, progesterone, testosterone, cortisol, IL-1, IL-6 and IL-10 levels in female and male subjects studied for *CYP* activity. To correlate the activity of *CYPs 1A2, 2D6* and 3A4 with circulating levels of cytokines and/or circulating levels of steroid hormones. To statistically assess the impact of each of the measured variables (race, age, gender, cytokine levels, hormone levels) on the *CYP2D6* phenotype and *CYP 1A2* and *CYP3A4* activity levels.

Accomplishments: Thus far, the Division has initiated recruitment of eight subsets of 20 individuals each that represent both young and old men and women, and in subgroups of women who are pregnant or taking oral contraceptives (OCs) or hormone replacement therapy (HRT). Blood and urine samples are being collected for hormonal analysis, cytokine measurements, and *CYP* activity. Statistical analysis will include evaluation of the interaction of hormonal/cytokine levels and specific *CYP* activities.

Phase I of this study funded through the OWH in FY98 required the recruitment of 160 participants for assessment of hormonal and cytokine levels and *CYP1A2* activity. This study is underway with 40 individuals pre-screened and enrolled in the study. Phase I will continue as Phase II is initiated.

Phase II will expand on this study by recruiting an additional 20 pregnant volunteers in order to add a ninth subgroup to the overall set of participants and will add assays to assess hormonal and cytokine interactions with *CYP3A4* and *CYP2D6*.

Four sample-sets on 25 volunteers have been completed (100 samples). Additionally, 11 individuals have repeated the study with individual drug regimens as well as combination drug regimens (total of 62 additional samples) to validate lack of drug-interaction with two probe drugs used in assessment of metabolic activity. An additional 40 individuals are registered in the database waiting to start the study.

Expected Agency Impact: The potential impact of the use of human-specific *in vivo* model systems to identify measurable markers that reflect differences in gender-related metabolic activity, such as the cytokines, would more safely and scientifically achieve the goals of both FDA and the pharmaceutical manufacturers in defining better indices of metabolic status for determining toxicity, dose, and efficacy of new therapeutic drugs.

Experiment No: E6894.01
Title: Chemoprevention of DNA Adducts of PhIP
Principal Investigators: Fred F. Kadlubar, George Hammons, Beverly Lyn-Cook, Daniel A. Casciano

Agency Benefit: The development of intervention strategies to protect against the carcinogenicity of the foodborne heterocyclic amines.

Specific Objectives: To examine a variety of putative chemoprevention regimens to inhibit PhIP-DNA adduct formation using the rat model of PhIP-induced colon and pancreatic carcinogenesis; and to elucidate the mechanisms of action of these agents in order to assess their applicability to human intervention trials.

Accomplishments: The coffee lipids, kahweol and cafestol, and green and black teas were the strongest inhibitors of PhIP-DNA adduct formation. Kahweol and cafestol were subsequently found not only to induce GSTs but also down-regulate NATs, effectively changing the phenotype of rats from rapid to slow acetylators. This was further validated in experiments with rat hepatocytes and similar studies are nearly

complete using human hepatocyte preparations. In addition, quercetin, ethoxyquin, tannic acid, green tea and black tea were found to strongly inhibit the *N*–oxidation of PhIP and ABP in human liver microsomes with the tea theaflavins showing the most potent effect. Black and green tea extracts were also found to strongly inhibit cell growth in both pancreatic and prostate tumor cells. In the pancreatic cell line, black and green tea decreased the expression of the *K-ras* gene and the multi-drug resistant gene *(mdr-1). R*esults demonstrate that components of coffee and of black and green teas can inhibit carcinogen bioactivation and also modulate the expression of genes known to play a role in the carcinogenesis process.

Expected Agency Impact: These results indicate the need for human studies on the impact of tea and coffee consumption on drug bioavailability, adverse drug reactions, and on colon and urinary bladder cancer risk by foodborne and environmental heterocyclic/aromatic amine carcinogens.

ACTIVE PROJECTS FY1999

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0689401	**Chemoprotection of DNA Adducts of 2-Amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine in the Rat** To examine the effect of the glutathione S-transferase inducers, phenethylisothiocyanate, diallyl sulfide (DAS), 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (Oltipraz), garlic powder, cabbage powder, 2(3)-tert-butyl-4-hydroxyanisole (BHA), kahweol palmitate, cafestol palmitate, quercetin, tannic acid, a-angelicalactone, Green tea, and ethoxyquin on the metabolism and DNA adduct formation of the food-borne carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-b]- pyridine, in the Fischer 344 rat.	Teitel, Candee H.* Kadlubar, Fred F. Lin, Dongxin
E0694601	**A Case-Control Study of Pancreatic Cancer & Aromatic Amines** To measure the associations of aromatic amine exposure and metabolism with the risk of pancreatic cancer. The sources of aromatic and heterocyclic amines to be studied are cigarette smoking and diet; the metabolic capabilities to be studied are acetylator status and N-oxidation status.	Kadlubar, Fred F.* Anderson, Kristen Potter, John D.
E0694701	**Role of Acetylation & N-Oxidation in Colorectal Cancer** To confirm the initial findings of our pilot study regarding the roles of heterocyclic amine metabolism and exposure as putative risk factors from the diet or the environment. The sources of heterocyclic amines to be studied are cigarette smoking, diet and cooking methods; the metabolic pathways to be studied include heterocyclic amine N-oxidation status and O-acetylation status.	Kadlubar, Fred F.* Lang, Nicholas P.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0696201	**Methylation Profile, Gene Expression, and Enzyme Activity of CYP1A2 in Human Livers** This protocol will serve as a preliminary study to determine the possible involvement of epigenetic mechanisms in the regulation of the expression of the CYP1A2 gene. The methylation status determined for each sample will be correlated with the expression of the CYP1A2 gene and enzyme activity.	Hammons, George J.* Blann, Ernice Kadlubar, Fred F. Lyn-Cook, Beverly A.
E0697801	**Chemical Carcinogenesis: Epithelial Cells in Breast Milk** 1. To develop and refine a methodology for separation of luminal epithelial cells from human breast milk for DNA extraction; 2. To detect and quantify aromatic/hydrophobic-DNA adducts in luminal epithelial cells derived from human breast milk; 3. To detect genetic polymorphisms in carcinogen-metabolizing genes derived from DNA extracted from epithelial cells in human breast milk; 4. To evaluate the relationships between carcinogen-DNA adducts and smoking status, and adduct levels with polymorphisms in NAT1, NAT2, CYP1A1, and GSTM1.	Ambrosone, Christine B.* Kadlubar, Fred F. Tang, Yong M. Thompson-Carino, Patricia
E0698101	**Investigation of Short Term Dietary Methyl Supplementation in Manipulation of DNA Methylation and Methyl Metabolism in Mice** To determine whether short term dietary methyl supplementation in mice will effect qualitative or quantitative changes in levels of methyl metabolites, levels of DNA methylation or levels of cell proliferation or apoptosis. The effects will be determine at two time points and at three levels of methyl supplementation. The studies proposed herein will provide data on some molecular and cellular events resulting from methyl supplemented diets. These studies will provide specific new data and use new test strategies that will help us better extrapolate between human and animal data.	Poirier, Lionel A.*
E0699001	**The Role of Human Cytochrome CYP1B1 in Drug Metabolism and Carcinogenesis** To elucidate the role of human cytochrome P450 IB1 (CYP1B1) in drug metabolism and carcinogenesis. Specific aims are: To design and develop peptide-specific antibodies against human CYP1B1; Determine the levels of CYP1B1 protein in various human tissues; Evaluate CYP1B1 expression as a biomarker for tumorigenesis; Identify CYP1B1 inducers among the most common drugs and carcinogens; Identify CYP1B1 substrates, including the endogenous steroid hormones, as well as drugs and carcinogens known to be metabolized by the closely related cytochromes P450 IA1 and IA2; Find specific enzyme inhibitors for CYP1B1; develop a sensitive, convenient, and specific assay method for CYP1B1 enzyme activity *in vitro*; Evaluate genetic polymorphism(s) for CYP1B1 as an epidemiological marker for cancer.	Tang, Yong M.* Kadlubar, Fred F.
E0699011	**ADDEND: The Role of Human Cytochrome CYP1B1 in Drug Metabolism and Carcinogenesis** Requesting to add *in situ* hybridization as an additional approach to investigate the expression of CYP1B1 in various human tissues. This will be performed in addition to the immunohistochemistry of the protocol. Requesting inclusion of Pathology support in the performance of these studies.	Tang, Yong M.*

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0699501	**Characterization of Ovarian-Specific Biotransformation of Estradiol: A Model for the Identification of Inter-individual Variability in Tissue-Specific Steroid Metabolism** With the current widespread use of hormone-based therapies and the increasing support for hormone-based chemoprevention therapies for breast cancer, concern regarding the role of estrogens, anti-estrogens and progesterones in the etiology of and/or progression towards cancers of hormonally-responsive tissues has continued to remain controversial in the cancer literature. Numerous studies, both epidemiological, as well as animal exposure studies, strongly suggest a role for estrogens in the carcinogenic cascade of several hormone-responsive cancers. It is predicted that the identifcation of genetic variablity in estrogen metabolism among individuals can be utilized as biomarkers to assess cancer risk in large population-based epidemiological studies, providing a tool to address more directly concerns regarding the association of estrogens/estrogen-metabolites, hormonal-based therapeutics and carcinogenesis in the human population.	Thompson-Carino, Patricia* Ambrosone, Christine B. Hamilton, Lea P. Kadlubar, Fred F. Tang, Yong M.
E0701501	**Breast Cancer in African-American Women: Metabolic Modification of Dietary and Hormonal Risk Factors** In this study, we intend to examine the role of interindividual variability in response to exogenous agents as it may relate to breast cancer risk in African-American women. By evaluating risk associated with exposure to oral contraceptives, hormone replacement therapy, and modification of that risk by genetic variability in their metabolism, the effects of substances regulated by the FDA on breast cancer risk in African-American women may be further elucidated. Additionally, a successful model to increase African-American participation in research studies would greatly assist in future studies related to FDA regulated substances in African-American populations.	Ambrosone, Christine B.* Stone, Angie Thompson-Carino, Patricia
E0701511	**ADDEND: The Role of Glutathione S-transferasse genetic Polymorphisms in Breast Cancer Sensitivity to Radio- and Chemotherapy** 1. To determine expression of enzymes (phenotype) in tumor tissue from women who recieved adjuvant therapy for breast cancer, using biopsy or surgical tissue specimens, using immunohistochemistry, and to evaluate associations between phenotypes in tumor tissue and risk of breast cancer recurrence; 2. To determine inherited GSTM1, GSTT1 and GSTP1 genotypes in normal tissue from these same women, and to determine associations of GSTM1, GSTT1 and GSTP1 genotype with phenotype in tumor tissue; 3. To evaluate if GST genotypes predice breast cancer recurrence following treatment, controlling for other factors that may relate to prognosis.	Ambrosone, Christine B.* Coles, Brian F. Stone, Angie
E0701701	**The Effects of Low Zinc Levels on Ras, Mdr-1 Gene Activation and on Metabolic Enzyme Activities in Normal and Neoplastic Human Pancreatic Cells: A Possible Risk Factor for Pancreatic Cancer** The major objective of this proposal is to determine the effects of nicotine and other cigarette components on exocrine and endocrine human pancreatic cells *in vitro*. The final objective of this study is to examine *ras*, mdr-1, CYP1A1 and CYP1A2 expression in normal and neoplastic human pancreatic tissue grouped according to race and sex obtained from a human tissue bank.	Lyn-Cook, Beverly A.* Blann, Ernice Hammons, George J. Kadlubar, Fred F.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0702101	**Prostate Cancer: Exposure, Susceptibility and DNA Adducts** Specific Aim 1: Determine levels of carcinogen exposure in African Americans and Caucasians with histologically confirmed prostate cancer using a case-control design; Specific Aim 2: Evaluate variability in hormone metabolism and susceptibility to carcinogen exposure, as measured by phenotypic and genotypic variability in carcinogen metabolism, and evaluate the interaction of these factors with the exposure data obtained in Specific Aim 1; and, Specific Aim 3: Characterize DNA adducts in prostate tissue from men with prostate cancer to identify mutagenic agents and evaluate levels of adducts in relation to carcinogen exposure data and susceptibility factors obtained in Specific Aims 1 and 2.	Ambrosone, Christine B.* Green, Bridgett L. Hine, R. Jean Kadlubar, Fred F. Lang, Nicholas P. Stone, Angie Thompson-Carino, Patricia
E0704301	***In Vivo* Modeling of Steroid-mediated Gender Effects in Drug Metabolism** 1. To characterize the activity of CYP1A2 in female subjects with regard to age, race, phase of the menstrual cycle, pregnancy, oral contraceptive usage, menopause, and hormone replacement therapy (HRT); 2. To characterize the activity of CYP1A2 in male subjects with regard to age; 3. To measure estradiol, progesterone, testosterone, cortisol, Il-1, IL-6 and IL-10 levels in female and male subjects studied for CYP1A2 activity; 4. To correlate the activity of CYP1A2 with circulating levels of cytokines and/or circulating levels of steroid hormones; 5. To statistically assess the impact of each of the measured variables on the CYP1A2 phenotype.	Thompson-Carino, Patricia* Ambrosone, Christine B. Delongchamp, Robert R. Kadlubar, Fred F. Lang, Nicholas P. Macgregor, Jim
E0697811	**ADDEND: Chemical Carcinogenesis: Epithelial Cells in Breast Milk** 1. To measure the levels of aromatic amines in human breast milk; 2. To evaluate the mutagenicity of human milk and milk fat in an Ames *Salmonella* test highly sensitive to aromatic amines and; 3. To evaluate relationships between aromatic amines in milk, mutagenicity, and carcinogen-DNA adduct levels in ductal epithelial cells with exposure and susceptibility factors.	Ambrosone, Christine B.* Josephy, David Thompson-Carino, Patricia
E0704311	**ADDEND: Part II of *In Vivo* Modeling of Steroid-mediated Gender-effects in Drug Metabolism** 1. To determine the activity of CYP2D6 and 3A4 in female and male subjects with regard to age, race, phase of the menstrual cycle, pregnancy, oral contraceptive usage, menopause, and HRT; 2. Measure estradiol, progesterone, testosterone, cortisol, IL-1, IL-6 and IL-10 levels in female and male subjects studied for CYP activity; 3. Correlate the activity of CYP2D6 and 3A4 with circulating levels of cytokines and/or circulating levels of steroid hormones; 4. Statistically assess the impact of each of the measured variables on the CYP2D6 phenotype and CYP3A4 activity level.	Thompson-Carino, Patricia* Delongchamp, Robert R. Kadlubar, Fred F. Mcclure, Gail Y.
E0701521	**ADDEND: The Role of Glutathione S-transferase Genetic Polymorphisms in Breast Cancer Sensitivity to Radio- and Chemotherapy** Tumor tissue was to be retrieved, sliced and stained by collaborators in the Dept. of Pathology at the UAMS Medical Center. Staffing and personnel have changed in the Pathology Dept. however, and although they will slice the tumor blocks, they are no longer able to perform IHC for the GSTs. Requesting support from NCTR pathology staff for immunohistochemistry (IHC) staining of approximately 200 blocks for four GSTs.	Ambrosone, Christine B.*

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3. Gross, M., Anderson, K., Lang, N.P. and Delongchamp, R.R. The distribution and concordance of N-acetyltransferase genotype and phenotype in an American population. Cancer Epidemiology, Biomarkers and Prevention, 8:683-692, 1999. Accepted: 2/1/99. **(E0694601)**

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MICROBIOLOGY

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Dr. Saeed A. Khan uses molecular techniques to detect antibiotic resistance markers in bacteria.

INTRODUCTION

Microbiology is an exceptionally broad discipline encompassing research areas as diverse as taxonomy, physiology, biochemistry, molecular biology, pathogenesis, food and industrial microbiology, and ecology. In fact, modern biotechnology rests upon a microbiological foundation. The microbiology research at the NCTR serves a multipurpose function with specialized expertise to perform fundamental and applied microbiology research in areas of the FDA responsibility. The microbiology research also responds to microbial surveillance and diagnostic needs for research projects within the Agency. The major aims of the microbiology research program are to raise the general awareness of the importance of microorganisms in public health and to provide data to improve our understanding of the mechanisms by which various compounds are metabolized and toxic events occur in humans. The research is organized to handle many aspects of microbial toxicology and the staff is continually trained to meet the research and regulatory needs of the FDA. The microbiology research at NCTR is divided into five focal areas with strategies and objectives unique to the problem posed. These are: 1) foodborne pathogens, food safety, and methods development; 2) the determination of the role of intestinal microflora in the metabolism and activation or detoxification of xenobiotics; 3) environmental biotechnology; 4) the use of microorganisms as models to predict the metabolic pathways by which drugs are metabolized in mammals; and 5) microbiological surveillance and diagnostic support of research. Accomplishments and goals for each focal area are discussed separately below.

Foodborne Pathogen Research, Food Safety, and Methods Development.

Despite the fact that the United States food supply is the safest in the world, tens of millions of cases of foodborne illnesses occur in the United States every year with a cost to the economy of an estimated 1 to 10 billion dollars. Therefore, the microbiological safety of food has become an important concern of consumers, industry, and regulatory agencies. The FDA gives a high priority to protecting the public from microbial contamination of the food supply. The research program in the Division of Microbiology has developed molecular methods to detect and identify foodborne bacterial pathogens. In addition, scientists in the Microbiology division collaborate with

scientists in the Chemistry division to use mass spectrometry methods for the rapid identification of bacteria. Furthermore, a number of collaborative research projects have been initiated, based on the goals of the Food Safety Initiative.

In FY99, we collaborated with the Center for Veterinary Medicine (CVM), Center for Food Safety and Applied Nutrition (CFSAN), and the Office of Regulatory Affairs (ORA) on a variety of projects, which include: 1) development and/or evaluation of methods for the detection of human pathogens in the animal environment and feeds; 2) investigations of the microbiological consequences of the use of antibiotics in the animal production environment; 3) investigations of factors associated with the emergence, transmission, and carriage of human foodborne pathogens in or on food-producing animals and edible products derived from them; 4) methods to assess the effects of veterinary antimicrobials on the human intestinal microflora; and 5) the environmental fate of veterinary antimicrobials.

A project (E06988.01) for the detection of 13 species of foodborne pathogens in foods using the polymerase chain reaction (PCR) technique was completed in FY99. The method used a universal enrichment medium and the same PCR conditions with 13 sets of specific primers for the detection of foodborne pathogens. The foodborne pathogens examined were *Escherichia coli, Shigella, Salmonella, Yersinia enterocolitica, Y. pseudotuberculosis, Vibrio cholerae, V. parahaemolyticus, V. vulnificus, Listeria monocytogenes, Staphylococcus aureus,* and *Bacillus cereus*. No interferences were observed while using the PCR assay for food samples artificially inoculated with each single bacterial species.

In collaboration with the Division of Chemistry (E06932/E07005.05), rapid methods of identifying whole bacteria by their mass spectra were developed. Rapid identification is needed for FDA inspectors to make quick decisions on whether to allow the sale of food products that may harbor pathogenic bacteria. Bacterial proteins that are prominent in the MALDI TOF mass spectra from whole cells of *Escherichia coli, Shigella flexneri, Pseudomonas aeruginosa,* and *P. putida* were identified and sequenced. The proteins from *E. coli* and *S. flexneri* were the acid-resistance proteins HdeA and HdeB; the protein from *P. aeruginosa* was the cold-shock protein CspA, and the protein from *P. putida* was the cold-acclimation protein CapB.

A collaborative project (E07001.01) was initiated with the FDA Gulf Coast Seafood Laboratory, Dauphin Island, AL, on why the supernatant fraction of centrifuged oyster homogenate is lethal to *Vibrio cholerae* and *V. vulnificus*. While cholera occurs primarily in developing countries, it is a major concern for FDA because the U.S. imports food from countries with endemic cholera. Toxigenic *V. cholerae* was found in oysters in Mobile Bay by both FDA and the Centers for Disease Control and Prevention (CDC). The lethal activity of the oyster homogenate to *V. cholerae and V. vulnificus* led to the isolation of *V. vulnificus*-specific bacteriophages. Several of these phages were isolated and a few of them had a broad host range. One of these phages was purified by ultra-centrifugation and characterized These phages could be used to develop a phage-luciferin-luciferase or phage-green-fluorescent-protein based reporter system to detect

the pathogenic *V. vulnificus* in seafoods. However, since there is not enough biochemical and genetic information available about these phages, their genetic and biochemical characterization will be necessary to develop such a reporter system.

Erythromycin is extensively used for the control of staphylococcosis. The prevalence of the erythromycin-resistance genes (*erm*) in *Staphylococcus* spp. isolated from diseased chickens was determined. Forty-six (46) erythromycin-resistant *Staphylococcus spp.* were isolated from diseased chickens. A majority of the isolates were resistant to high concentrations of erythromycin, oleandomycin, spiramycin, and tylosin. Thirty-four (34) of these isolates were coagulase-positive *S. aureus* and the remaining 12 were coagulase-negative. Dot-blot hybridization indicated that ten of the 12 coagulase-negative strains harbored the *erm*A gene. Similar analysis also indicated that 22 of the 34 coagulase-positive *Staphylococcus spp.* harbored the *erm*A gene. The *erm*A gene was exclusively found on the chromosome. Two different ermA *EcoR*1 restriction-fragment-length polymorphisms were identified. A majority of the ermA- positive strains had two ermA inserts identified as 8.0 and 6.2 kb EcoR1 fragments. A few strains had 6.4 and 5.8 kb *EcoR*1 fragments. Plasmids (2.0-16.0 kb) were present in all the isolates. Southern hybridization indicated that only two of the 12 coagulase-negative *Staphylococcus spp.* contained the *erm*C gene on the plasmid. Twelve (12) of the 34 strains of *S. aureus* contained the *erm*C gene. Eleven (11) of these strains had the ermC gene on a 2.5 Kb plasmid and one strain had the gene on a 4.0 kb plasmid. Results indicate that either *erm*A or ermC was present in all isolates and that *erm*A was the dominant gene in coagulase-negative and coagulase-positive avian *Staphylococcus spp.* The resistant determinants were mostly *erm*A and *erm*C. Both of these determinant genes were transferable to clinica *S. aureus* strains. The mechanism of drug-resistance transfer was transposition and transposon-assisted plasmid mobilization.

Competitive exclusion products have the potential to eliminate or reduce the use of antibiotics in poultry husbandry for prevention of colonization of birds by *Salmonella sp*. The law requires that all components of veterinary drugs, including competitive exclusion products, be defined. It is also desirable that mixed bacterial cultures applied to poultry for internal colonization not contain human pathogens or antibiotic-resistant bacteria. The Division developed a protocol with CVM (E07049.01) for isolation and identification of the bacteria in competitive exclusion cultures, using the most reliable phenotypic and genotypic microbial identification techniques available. Colonization of poultry with pathogenic or antibiotic-resistant bacteria can introduce these bacteria into the human food supply when the poultry are processed. Preliminary results have already alerted CVM to the possibility that competitive exclusion products can introduce bacteria with undesirable antibiotic-resistance into the human food supply. Producers of competitive exclusion products will need guidelines to submit reliable information to the FDA for product approval. The Division has also been evaluating a cell culture model that can determine whether a competitive exclusion product can effectively exclude *Salmonella* sp. or other invasive bacteria from intestinal cells. This assay will be available to producers and the FDA to evaluate the efficacy of competitive exclusion products.

Another project (E07053.01), developed in collaboration with CVM, involves the assessment of the potential of a competitive exclusion product to spread vancomycin resistance. Resistance to vancomycin was unknown until 1988, but the isolation of vancomycin-resistant bacteria is increasing every year. Vancomycin-resistant bacteria are usually multidrug-resistant and do not respond to any antimicrobial therapy. The use of vancomycin in humans and avoparcin in cattle has resulted in vancomycin-resistant bacteria. It is important to monitor and control the spread of vancomycin resistance. The competitive exclusion product is a mixture of 29 different bacteria. Eleven (11) of these bacteria are highly resistant to vancomycin and ten of them belong to the genus *Lactobacillus.* Some species of *Lactobacillus* spread vancomycin resistance, and one of these species is present in the competitive exclusion product. Its presence could contribute to the transfer of resistance to other bacteria in chickens that may then be passed on to human beings. A multiplex-PCR assay to simultaneously detect the vancomycin-resistance-determinant genes, *VanA*, *VanB*, *VanC1*, *VanC2* and *VanC3* in a single reaction tube was developed to monitor the spread of these genes.

FY2000 GOALS

- Study the mechanisms of fluoroquinolone-resistance in *Salmonella* spp. isolated from poultry feeds and the production environment, and develop molecular methods for screening the drug-resistance genes. **[E07048.01]**

- Study the mechanisms of multi-drug-resistance in *Salmonella typhimurium* DT104 strains isolated from clinical, food, and environmental sources. **[E07048.01]**

- Establish molecular screening methods for the determination of vancomycin resistance in bacteria from the selective competitive exclusion product DF3 (Preempt™). **[E07053.01]**

- Assess the vancomycin-resistant bacteria in the Preempt™ product for their potential to transfer the resistance to clinical *Staphylococcus aureus* isolates. **[E07053.01]**

- Conduct a comparative analysis of erythromycin-resistance-determinant genes in poultry, bovine, and clinical staphylococcal isolates. **[E06901.00]**

- Conclude research work on a broad host range *Vibrio vulnificus* phage, 71A-6. **[E07001.01]**

- Study the fluoroquinolone resistance in *Campylobacter* spp. isolated from poultry. **[E07050.01]**

- Determine the feasibility of assembling a pyrolysis mass spectrometric library for rapid chemotaxonomy of bacteria. **[E06931.01]**

- Characterize bacteria in aqueous media by MALDI TOF mass spectrometry of isolated biomarker proteins. **[E07005.05]**

- Conclude the evaluation of *in vitro* efficacy of competitive exclusion products and the phenotypic and genotypic analyses of competitive-exclusion bacteria. **[E07049.01]**

- Conclude the evaluation of the antimicrobial sensitivities of competitive exclusion product bacteria. **[E07049.01]**

- Adapt an *in vitro* competitive exclusion assay to measure antibiotic residue concentrations that perturb colonization resistance by enteric flora.

- Perform an evaluation of the FDA Bacteriological Analytical Manual (BAM) cultural and molecular methods to identify foodborne pathogens in food, feed, and animal production environments, including feces. **[E07051.01]**

The determination of the role of intestinal microflora in the activation or detoxification of xenobiotics.

A second subject that has received much attention and emphasis in the Division of Microbiology is the importance of intestinal microflora in the metabolism of antimicrobial compounds, food additives, and food supplements. Rapid molecular biology techniques have been developed to detect intestinal microflora population changes following exposure to these xenobiotics. The question of whether the use of antimicrobials in food-producing animals will lead to the emergence of antimicrobial resistance in microbes infecting humans has been a controversial topic of debate at scientific meetings for over 30 years. To assess the safety of ingested antibiotic residues to the consumer, national and international committees have evaluated data on the chemical, pharmacological, toxicological, and antimicrobial properties of veterinary drugs, based on studies of experimental animals and observations in humans. In response to CVM's need for assessing the microbiological safety of animal drug residues in food, the Division of Microbiology will be examining *in vitro* models for testing the effects of low levels of antimicrobial residues on the human intestinal microflora this fiscal year.

The conversion of natural isoflavonoids to compounds with high estrogenic activity by anaerobic bacteria from the human intestinal tract was investigated (E07007.01). Isoflavonoids are naturally occurring dietary phytoestrogens, present in different plants, especially from the legume family. The phytoestrogens and their metabolites have been proposed for use in the prevention and therapy of hormone-dependent diseases and their availability and consumption have increased in recent years. The principal plant isoflavonoids, genistein and daidzein, also have been found in their methylated forms, biochanin A and formononetin, respectively. The estrogenic potencies of genistein and daidzein are substantially higher than those of methylated forms. The bacteria from the human intestinal microflora have different enzymatic activities, with a variety of metabolic functions, including *O*-demethylation of various compounds. *Eubacterium limosum*, an anaerobic bacterium from the human intestinal tract, is known to *O*-demethylate methoxylated benzoic acids and was assayed for the *O*-demethylation of

biochanin-A and formononetin. Following anaerobic growth of this bacterium with these compounds, genistein and daidzein were produced, as shown by the mass spectral analysis of metabolites detected and purified by high-performance liquid chromatography. This was the first bacterium from the human intestinal microflora to be shown to convert natural isoflavonoids to estrogenic metabolites.

The effects of norfloxacin on the development of resistance in bacteria from the human GI tract and on the modification of the antimicrobial activity of norfloxacin by resistant bacteria were evaluated. Norfloxacin is used for the treatment and prophylaxis of a variety of bacterial infections. This results in the development of resistant bacteria. Several anaerobic bacteria that had developed resistance to high concentrations of norfloxacin were isolated. The effects of resistant bacteria on the antimicrobial activity of norfloxacin were evaluated by a bioassay using norfloxacin-susceptible *Escherichia coli*. The filter-sterilized supernatants of norfloxacin incubated with the resistant bacterial cultures did not inhibit the growth of *E. coli*, indicating that the anaerobic bacteria had modified norfloxacin to compounds without antimicrobial activity. Two of the bacteria resistant to norfloxacin also were resistant to ciprofloxacin and ofloxacin. This study shows that human intestinal bacteria ordinarily sensitive to norfloxacin can become resistant to high norfloxacin concentrations; then, metabolize it and decrease its effectiveness against norfloxacin-susceptible strains.

FY2000 GOALS

- Evaluate the effects of veterinary drug residues on intestinal microflora in a semicontinuous culture system.

- Detect metabolic pathways in the conversion of phytoestrogens (daidzein and genistein) to estrogenic and nonestrogenic end products by bacteria from the human intestinal tract. **[E07007.01]**

- Determine the mechanism of resistance to fluoroquinolones in bacteria from the human intestinal tract.

- Develop molecular techniques based on terminal restriction fragment patterns, for the detection of the predominant anaerobes of human intestinal flora.

- Develop a competitive exclusion model to assess the effects of veterinary antimicrobials on the human intestinal microflora.

Environmental Biotechnology

Historically, a major focus in the environmental biotechnology area in the Division of Microbiology has been to determine the biodegradation of a wide range of pollutants, with special emphasis on the ubiquitous carcinogens, polycyclic aromatic hydrocarbons (PAHs). Fundamental and applied studies on the biodegradation pathways and on the enzyme and genetic bases for biodegradation of priority pollutants have been conducted. Currently, in collaboration with investigators at CVM, the microbial transformation of veterinary antimicrobial drugs is being determined.

Bioremediation principles, *i.e.*, the use of microorganisms to degrade pollutants under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities, offers great promise for accelerated removal of chemical pollutants in the environment. A drug registration package must contain data that demonstrate the proposed substance is efficacious against target pathogens, safe for human use, and safe for the environment.

A project (E06901.01) was developed in collaboration with the CVM to evaluate the environmental impact of antimicrobials and feed additives used in fish-farming systems. Antimicrobials are used extensively around the world for control of fish diseases. Currently, the antibiotic erythromycin is under FDA's review for approval for use in salmon and trout culture, specifically for control of bacterial kidney disease. Since aquaculture wastewater and sediment are discharged into the environment, there is concern over the potential detrimental effects on the environment and public health. CVM needs environmental impact and biological activity data on erythromycin before any approval for aquaculture.

Few studies have reported on the environmental fate of erythromycin used in aquaculture. NCTR scientists in collaboration with The Bionetics Corporation were successful in biosynthesizing [C^{14}]-erythromycin. To monitor erythromycin in aquaculture samples, a quick, easy, and reliable detection method was required. An indicator bacterium sensitive to 0.05 μg/ml of erythromycin was isolated from the aquaculture pond sediments and was identified by molecular methods, including 16S rRNA, biochemical profiles, fatty acid analysis, and PCR techniques, as a *Stenotrophomonas* species. Using this new indicator organism, the NCTR scientists were successful in developing a sensitive bioassay procedure to determine the biological activity of erythromycin in aquaculture samples. This technique is suitable for testing water from aquaculture ponds and marine environments, as well as extracts of a variety of sediment samples. This bioassay procedure is specific for erythromycin detection in the presence of other commonly used aquaculture drugs.

Using biometer flasks, mineralization of erythromycin (CO_2 evolution) was monitored for over 70 days at 5°C and 10°C after the addition of 20 μg/ml unlabeled erythromycin and [1,3,5,7,9,11,13-^{14}C]erythromycin. Sediments were collected from one fish hatchery that had used erythromycin during the past three years for control of disease in salmon. Sediments were also collected from another hatchery that had not received any

medicated feed for the past six years. The biodegradation studies indicated that more than 50% of the applied ^{14}C-erythromycin was mineralized to $^{14}CO_2$ in the first sediment at 10°C and 16% at 5°C, indicating the effect of temperature on the mineralization rates. For the second sediment, the mineralization rates were 45% and 30% for 10°C and 5°C, respectively. Presently, these sediment samples are also being monitored for mineralization rates using a flow-through microcosm system developed at NCTR, which closely mimics the natural ecosystem. Advantages of using this system include the mass balance accountability of the compound of study and the controlled environment.

The fluoroquinolones are widely used in veterinary and clinical medicine for treatment of bacterial infections. The large-scale use of fluoroquinolones in animals has become highly controversial because of the increased drug resistance among pathogenic bacteria; used poultry litter may contain pathogenic bacteria with resistance to 12 commonly used antibiotics. A strain of the saprobic fungus *Mucor ramannianus,* isolated from a forest, was used to demonstrate the potential for fluoroquinolone biotransformation by fungi in the environment. The fungus carried out the *N*-acetylation of ciprofloxacin to a single product, which was purified from culture extracts by HPLC. The metabolite was identified by mass and NMR spectrometry as *N*-acetylciprofloxacin.

The metabolism of enrofloxacin by *M. ramannianus* was investigated as a model for the biotransformation of veterinary fluoroquinolones by fungi. HPLC analysis showed three metabolites which were purified by HPLC and initially characterized by the UV absorption spectra. They were identified by mass and NMR spectrometry as enrofloxacin *N*-oxide, *N*-acetylciprofloxacin, and 1-cyclopropyl-7-{[2-(ethylamino)-ethyl]-amino}-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid. Thus, *N*-acetylciprofloxacin has now been identified as a fungal metabolite of both ciprofloxacin and enrofloxacin. The transformation of enrofloxacin by *M. ramannianus*, including *N*-oxidation, *N*-dealkylation, *N*-acetylation, and breakdown of the piperazine ring, is similar to the metabolism of fluoroquinolones in mammals. Since fungi are common in soil and decomposing organic matter, the biotransformations of drug residues by this group of fungi are likely to be ecologically significant.

FY2000 GOALS

- Determine the effect of poultry litter on biotransformation of fluoroquinolones by fungi. **[E07052.01]**

- Determine the environmental consequences of the use of veterinary pharmaceuticals in concentrated animal feedlot operations. **(EPA)**

- Determine the kinetics of erythromycin degradation in water and sediments. **[E06901.01]**

- Determine the potential of pure cultures of fungi to biotransform fluoroquinolones. **[E07052.01]**

The use of microorganisms as models to predict the metabolic pathways by which drugs are metabolized in mammals.

Another research initiative within the Division of Microbiology is to exploit the use of microorganisms as models of mammalian drug metabolism. Before any drug can be approved for use in humans, extensive studies are required to establish its efficacy and safety. The evaluation of drug metabolism constitutes an important and necessary step in this process. However, identification of metabolites from animal sources and structure/activity relationships can be hindered by insufficient quantities of materials. Therefore, this program has used microorganisms for the biotransformation of a wide range of drugs, including antihistamines, tricyclic antidepressants, and antibiotics, to provide sufficient metabolites, which mimic mammalian reactions, for structure elucidation and toxicity evaluation. Biochemical and molecular genetic studies on the phase I and phase II reactions involved in the microbial biotransformation of pharmaceutical compounds are currently being investigated.

Studies in the Division of Microbiology (E06942) have shown that the filamentous fungus *Cunninghamella elegans* simulates mammalian metabolism by phase I and phase II enzymes for numerous structurally diverse pharmaceutical compounds. *C. elegans* biotransforms alkylamine, ethanolamine and ethylenediamine-type antihistamines, such as triprolidine, pheniramine, diphenhydramine, and thenyldiamine; tricyclic antidepressants, such as doxepin, cyclobenzaprine, amitriptyline, and protriptyline; and phenothiazines, such as chloropromazine and methdilazine; to a range of different metabolites by aliphatic and aromatic hydroxylation, epoxidation, *N*- and *O*-dealkylation, and *N*- and *S*-oxidation reactions. These studies further demonstrate the usefulness of fungi to predict mammalian phase I drug metabolism and their potential for large-scale metabolite production for toxicological evaluation.

One example of the research on the use of fungi as a microbial model of mammalian drug metabolism is the biotransformation of cyclobenzaprine. Seventy-five percent (75%) of this drug, at a concentration of 1 mM, was metabolized by *C. elegans* within 72 h to milligram quantities of metabolites. The major metabolites were 2-hydroxycyclobenzaprine (59%), *N*-desmethylcyclobenzaprine (21%), cyclobenzaprine *trans*-10,11-dihydrodiol (5%), *N*-desmethyl-2-hydroxycyclobenzaprine (3%), 3-hydroxycyclobenzaprine (3%), and cyclobenzaprine *N*-oxide (1%). The isolation and characterization of these metabolites indicate that aromatic hydroxylation and *N*-demethylation are the major metabolic pathways used by *C. elegans*. Rat liver microsomes also qualitatively synthesize all of the six metabolites produced by *C. elegans*, which further supports the microbial model of mammalian metabolism.

Phenothiazines with N-carbonyl substituents have been used as antiarrhythmic drugs, coronary vasodilators, and antidepressants. Phenoxazine, a tricyclic xenobiotic compound, represents a structure found in the antibiotic dactinomycin and dyes such as Nile blue. Cultures of the fungi Aspergillus niger, Cunninghamella verticillata, and Penicillium simplicissimum, grown in a sucrose/peptone medium, transformed N-acetylphenothiazine to N-acetylphenothiazine sulfoxide and phenothiazine sulfoxide.

Phenothiazin-3-one and phenothiazine N-glucoside were also produced by *C. verticillata*. The probable intermediate, phenothiazine, was detected in cultures of P. simplicissimum. *Cunninghamella elegans* was grown in a medium containing phenoxazine and converted nearly all of it to a single metabolite, phenoxazin-3-one.

The fungal biotransformation of 9-nitroanthracene, a weak mutagen and a potential environmental pollutant, was studied. 9-Nitroanthracene was transformed by *C. elegans* to two major metabolites that were identified by HPLC, UV/visible spectrometry, mass spectrometry, and NMR spectroscopy as phenol and *trans*-dihydrodiol detoxification products. Similar biotransformation studies with vinclozolin, a dicarboximide fungicide used in several fruits and vegetables, showed formation of four metabolites hydroxylated on the side chain. Since vinclozolin is considered an environmental hormone disruptor, and is mediated by antiandrogenic metabolites in rats, the metabolites from the fungal metabolism studies will be collected and tested in rats for any such activity.

Although the Division continues to conduct further studies on the microbial transformation of pharmaceutically important compounds, emphasis has shifted to characterize the enzymes that catalyze these reactions.

The involvement of cytochrome P450 enzymes in the metabolism of pharmaceutical compounds by *C. elegans* was shown in previous studies on carbon monoxide difference spectra (absorption at 450 nm), cytochrome P450 inhibitors, and $^{18}O_2$ incorporation experiments and identification of a spectrum of metabolites that are similar to those formed by mammalian cytochrome P450 enzyme systems. In FY00, molecular biology techniques are being used to further characterize cytochrome P450 in *C. elegans*, since it is extremely difficult to purify microsomal proteins using conventional biochemical techniques. Since many of the phase I and phase II enzymes are localized in the microsomal fraction of the cell, the program will use a polyclonal antibody against *C. elegans* to screen a *C. elegans* cDNA library to clone the genes of the enzymes. Glutathione transferase from *C. elegans*, using protein biochemistry and molecular techniques, will be purified.

FY2000 GOALS

- Identify the products of phenoxazine metabolism by the fungus *Cunninghamella elegans*. **[E06942.01]**

- Conduct molecular cloning of the genes for phase I and phase II xenobiotic enzymes from *Cunninghamella elegans*. **[E06942.01]**

- Collect fungal metabolites of vinclozolin and test for hormone effects in rats.

Microbiological surveillance and diagnostic support of research.

Laboratory animals are susceptible to a wide variety of bacterial, viral, and parasitic infections, resulting in an altered animal model that consequently affects research and testing by introducing variables that confound results. Routine screening for infectious diseases assures reliable animal models and prevents costly, time-consuming delays of research that could affect FDA regulatory decisions. Studies utilizing animals depend on healthy test animals; therefore, it is NCTR's responsibility to maintain the best microbiological diagnostic laboratory possible. The investigators and the FDA should be able to depend upon NCTR to support their efforts. Research goals for this sub-program are: 1) establishing and maintaining pathogen-free animals; 2) culturing and identifying microbial contaminants for other projects and programs within NCTR and other FDA centers; and 3) developing and testing new methods in diagnostic microbiology for other FDA centers.

Bovine spongiform encephalopathy (BSE) is a fatal, progressive neurological disease in cattle. To avoid the spread of BSE from cattle to other animals and humans, a ban on feeding ruminants with cattle-derived protein is in force. A PCR method has been developed in an European laboratory for the detection of bovine materials in feed. This method could be used by the FDA to aid in the enforcement of the ban. Therefore, a method validation trial of the PCR method was conducted in the Division of Microbiology in collaboration with CVM and ORA laboratories. The results demonstrated that it is a rugged, reliable test, with relatively low rates of false positives and false negatives. At the same time, an improved PCR method, which can be completed in two hours and is easy to perform, was developed. The new method is rapid, simple, and consistent and a significant improvement over the original method, which needed 24 hours to complete a tedious, multi-step purification process. The net result of the validation effort will be the transfer of a reliable, rugged test to the FDA field laboratories that will aid enforcement of the ban on cattle feed containing rendered-bovine protein.

Numerous chemical agents are used to disinfect and sterilize medical instruments, such as endoscopes, that cannot be autoclaved. Endoscopes contain crevices and channels that are difficult to clean and can harbor bacteria. Many of the liquid chemical germicides on the market claim the ability to kill *Mycobacterium tuberculosis*, yet improperly washed and disinfected endoscopes have been linked to the transfer of this organism from tuberculosis patients to previously uninfected individuals. This has raised the concern that some of the disinfectants may not be fully effective under the prescribed conditions.

The FDA is preparing to evaluate the tuberculocidal activity of a large number of liquid chemical germicides. The NCTR Division of Microbiology has been instrumental in the preparation for this evaluation by developing the expertise required to perform the Association of Official Analytical Chemists (AOAC) tuberculocidal assay, clarifying and expanding the protocol for this assay, and training Office of Regulatory Affairs (ORA) personnel to conduct this assay at their own facilities.

The current methods for determining the tuberculocidal activity of disinfectants are difficult to perform, poorly reproducible, and require up to 90 days to obtain results. Scientists are implementing molecular methods (E06965.01) to both improve the sensitivity and accuracy of the test and shorten the time required for a definitive answer. Using a mycobacterial strain carrying the firefly luciferase gene, they have developed a rapid, quantitative method for determining tuberculocidal activity by detecting light produced by bacteria that survive exposure to disinfectants. Because this method does not require the mycobacteria to grow significantly after the disinfectant exposure, the assay time is reduced to one day--compared with several weeks to months for other tuberculocidal activity tests. This method has been tested against several different disinfectant chemistries and shows promise in greatly reducing the time required to test the large number of liquid chemical germicides on the market. NCTR scientists will continue to improve this method, screening different test organisms with better growth characteristics and testing a broad range of disinfectants.

FY2000 GOALS

- Test mycobacterial strains for compatibility with the bioluminescent tuberculocidal assay. **[E06965.01]**

- Screen environmental conditions that affect disinfectant function, such as pH, temperature, organic load, and water hardness, using a bioluminescent assay. **[E06965.01]**

- Survey NCTR animal colonies for *Helicobacter hepaticus* and *Helicobacter bilis* by polymerase chain reaction (PCR) and evaluate serum-based detection methods, such as ELISA and western blot. **[E00262]**

- Establish a pathogen-containment animal room for support of research on interactions between pathogens and animals. **[E00262]**

- Provide NCTR Veterinary Services and researchers with microbiological surveillance and diagnostic support to determine and maintain the health status of the animal colony. **[S00006]**

- Continue the development of molecular genetic identification methods for rodent pathogens. **[E00262]**

PUBLIC HEALTH SIGNIFICANCE

The Division of Microbiology seeks to continue and expand its scientific exchange and collaborative studies with colleagues at other FDA centers and field laboratories to anticipate their research needs and provide data to support regulatory activities of the Agency and public health. These studies include: 1) metabolism and toxicological effects of food additives, antimicrobials and macronutrients on the intestinal microflora; 2) microbial production of metabolites of toxicological and pharmaceutical interest; 3) environmental fate and effects of aquaculture chemicals and other priority pollutants; 4)

tuberculocidal disinfectant testing; 5) detection of foodborne biological hazards; 6) rapid and accurate detection methods for pathogens and toxins; and 7) microbial surveillance of experimental animals.

Many of the techniques currently in use within the microbiology research area are of value to other FDA centers and field laboratories. As communication and discussion of mutual research interests between NCTR staff and other FDA scientists increases, many new projects at the forefront of applied microbiology research will be developed. The laboratory's vision is to strive for scientific excellence and to strengthen the relevance of its research to the mission of the FDA. It will continue to maintain a world-class research program to solve current issues that face the FDA in the next millennium so the Agency can make sound, science-based regulatory decisions on microbiological issues relative to public health.

ACTIVE PROJECTS FY1999

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0690101	**Microbial Degradation of Drugs and Feed Additives Used in Fish Farming (Aquaculture)** To develop a standardized method to evaluate the biodegradation of drugs and feed additives used in fish farming (aquaculture). To determine the biodegradation rates and metabolic fate of the antibiotic erythromycin in aquaculture water and sediments.	Pothuluri, Jairaj V.* Cerniglia, Carl E. Eirkson, Charles Nawaz, Mohamed S.
E0695901	**Cloning and Characterization of the Genes Involved in the Metabolism of Nitro Compounds by *Mycobacterium sp.* Pyr-1** To understand the substrate specificity, cofactor requirement, and molecular characteristics of *Mycobacterium sp.* Pyr-1 nitroreductase and to determine the relationship of this enzyme to other microbial and mammalian nitroreductases involved in reduction of therapeutic nitro compounds.	Rafii, Fatemeh* Cerniglia, Carl E. Hehman, Gery L.
E0700101	**Purification and Characterization of Antibacterial Protein from Oysters** 1. Purification of the antibacterial protein from oyster homogenate; 2. Physical, biochemical, immunological and molecular characterization of the protein; 3. Determination of the kinetics of the inhibitory reaction.	Nawaz, Mohamed S.* Cerniglia, Carl E. Depaola, Angelo Khan, Ashraf A.
E0700701	**Importance of Human Intestinal Microflora in Conversion of Phytoestrogens to Estrogenic Compounds** 1. Detection of various metabolites of phytoestrogens, produced by the metabolism of these compounds by pure culture of bacteria typical of that isolated from human microflora, and elucidation of the metabolic pathways of phytoestrogens by human intestinal bacteria; 2. Assessment of the estrogenic effect of each phytoestrogen metabolite produced by intestinal bacteria; 3. Determination of the bacterial species producing estrogenic metabolites from phytoestrogens and elucidation of enzymes involved in various steps of these metabolic processes; 4. The effects of phytoestrogens and their metabolites on the population, composition, metabolic activity and enzyme production of bacteria from the human gastrointestinal tract.	Rafii, Fatemeh* Cerniglia, Carl E. Sutherland, John B.

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0704801	**Studies on Mechanism of Fluoroquinolones Resistant *Salmonella* spp. Isolated from Animal Feeds (Poultry), Animal Production Environment and the Development of Molecular Methods for Screening the Drug Resistance Genes** 1. Isolation, identification and characterization of nalidixic acid and fluoroquinolone resistant *Salmonella* spp. from chicken farms (animal feed, feces, manure, litters and animals) by biochemical and Polymerase Chain Reaction; 2. Determination of minimum inhibitory concentration for environmental isolates, development of molecular techniques and its comparison with clinical strains; 3. Determination of drug resistance mechanisms in the environmental isolates and their characterization by molecular techniques; 4. Determination of influence of seasons and the frequency of isolation of fluoroquinolone-resistant *Salmonella* spp.	Khan, Ashraf A.* Cerniglia, Carl E. Gilbert, Jeffrey M. Jones, Roger A. Khan, Saeed A. Nawaz, Mohamed S. Summage-West, Christine V.
E0704901	***In Vitro* Model and Molecular Analysis of Competitive Exclusion Products** 1. Evaluate individual component bacteria in a defined competitive exclusion (CE) product for exclusion of enteric pathogens from Caco-2 and CRL-2117 cell monolayers; 2. Define the antimicrobial susceptibility patterns of the component bacteria using Minimal Inhibitory concentration measurements; 3. Sequence analysis of 16s rRNA Polymerase Chain Reaction (PCR) products from defined culture component bacteria and development of a database containing the sequences for use in subsequent identification of the organisms in undefined CE products; 4. Application of the 16s rRNA sequence analysis procedure to detect and identify effective CE component bacteria in undefined CE products.	Wagner, Robert D.* Cerniglia, Carl E. Holland, Michael A. Jones, Roger A.
E0705001	**Studies on the Fluoroquinolone Resistance in *Campylobacter* sp. Isolated from Poultry** 1. Isolation and identification of fluoroquinolone-resistant *Campylobacter jejuni* and *C. coli* from water, feed and litter samples in poultry houses; 2. Determination of the optimum concentration of nalidixic acid and fluoroquinolone resistance in *C. jejuni* and *C. coli*; 3. Determination of the influence of various seasons and the frequency of isolation of fluoroquinolone-resistant *C. jejuni* and *C. coli*; 4. Molecular characterization of fluoroquinolone resistance by polymerase chain reaction (PCR), nucleotide sequencing and single-strand conformation polymorphism (SSCP).	Nawaz, Mohamed S.* Cerniglia, Carl E. Gilbert, Jeffrey M. Jones, Roger A. Khan, Ashraf A. Khan, Saeed A. Pothuluri, Jairaj V. Steele, Roger S.
E0705301	**Molecular Screening Methods for the Determination of Vancomycin Resistance in Selective Competitive Exclusion Product CF3 (PREEMPT) Bacteria** 1. Islolation, identification and biochemical characterization of vancomycin resistant bacteria present in a commercially available competitive exclusion product CF3; 2. Development of a rapid PCR method of the detection of vancomycin resistance determinant genes, namely, the Van A0, Van B, Van C and D-ala-D-lac ligase gene Ddl.; 3. The characterization of plasmid DNA Profile and plasmid-mediated drug resistance transfer; 4. Genetic fingerprinting of the vancomycin resistant microorgnaisms present in PREEMPT culture; 5. Nucleotide sequence analysis of the PCR products of vancomycin resistant determinant genes showing interesting restriction profiles.	Khan, Saeed A.* Cerniglia, Carl E. Jones, Roger A. Khan, Ashraf A. Nawaz, Mohamed S.

PROJECTS COMPLETED FY1999

Project Number	Title/Objective	Principal*/ Co-Principal-Investigator(s)
E0696501	**Development of an Improved Method for Determining the Tuberculocidal Activity of Chemical Disinfectants for Medical Devices** To develop an improved method for the rapid and accurate evaluation of the tuberculocidal activity of chemical disinfectants and sterilants. The hypothesis is that molecular methods can be used to (a) improve quantification of the disinfectant activity, (b) improve the reliability of the assay, and (c) shorten the time required for testing in comparison with the standard culture techniques. This protocol addresses the NCTR strategic research goal to conduct method-, agent-, or concept-driven research, through satisfying the need for an analytical method to accurately evaluate these products.	Erickson, Bruce D. * Campbell, Warren L. Holland, Michael A.
E0699901	**Biochemical and Molecular Analysis of Polycyclic Aromatic Hydrocarbon (PAH) Degradation by Bacteria** 1. To characterize multiple genes for the initial aromatic dioxygenase from F. yanoikuyae B_1; 2. To determine putative common roles of ferredoxin and reductase components of initial dioxygenase in mono- and polycyclic aromatic hydrocarbon degradation; 3. To determine roles of the NahD (2-hydroxychromene-2-carboxylate isomerase) and NahE (cis-o-hydroxybenzylidenepyruvate aldolase) in polycyclic aromatic hydrocarbon degradation by S. yanoikuyae B_1; 4. To determine molecular basis for polycyclic aromatic hydrocarbon degradation by Mycobacterium sp. PYR-1.	Kim, Eungbin * Cerniglia, Carl E. Pohland, Albert
S00189	**Tuberculocidal Efficacy of Various Disinfectants** This support number established to take the place of E06793.00 - Assess, modify and validate the AOAC tuberculocidal test procedures for use with disinfectants.	Holland, Michael A. * Chamberlain, Virginia

FY1999 PUBLICATIONS*

1. Assaf, N.A., Pothuluri, J.V., Wang, R., Moffitt, C. and Cerniglia, C.E. Bioassay procedure for the evaluation of erythromycin activity in aquaculture environments. J. of the World Aquaculture Society, 30:137-146, 1999, Accepted: 11/27/98. **(E0690101)**

2. Bezalel, L., Hadar, Y. and Cerniglia, C.E. Degradation of polycyclic aromatic hydrocarbons by the white-rot fungus *Pleurotus ostreatus*. Advances in Biotechnology, pgs. 405-421, 1998, Accepted: 10/1/98. **(NA)**

3. Cerniglia, C.E., and Kotarski, S. Evaluation of veterinary drug residues in food for their potential to affect human intestinal microflora. Regulatory Toxicology and Pharmacology, 29:238-261, 1999, Accepted: 3/30/99. **(E0681000)**

4. Duhart, B.T., Zhang, D., Moody, J.D., Freeman, J.P. and Cerniglia, C.E. Biotransformation of protriptyline by filamentous fungi and yeasts. Xenobiotica, 29:733-746, 1999. Accepted: 7/16/99. **(E0694201)**

* ***(_ _ _ _ _ _ _ _) Indicates the related NCTR project number; NA-Not Applicable***

5. Holland, R.D., Duffy, C.R., Rafii, F., Sutherland, J.B., Heinze, T.M., Holder, C.L., Voorhees, K., Lay, J.O. Identification of bacterial proteins observed in MALDI TOF mass spectra from whole cells. Analytical Chemistry, 71:3226-3230, 1999. Accepted: 5/5/99. **(Collaborating with Chemistry) (E0700501)**

6. Khan, A.A., Nawaz, M.S., Khan, S.A. and Cerniglia, C.E. Identification of *Aeromonas trota* (hybridization group 13) by amplification of the aerolysin gene using polymerase chain reaction. Molecular & Cellular Probes, 13:93-98, 1999. Accepted: 11/16/98. **(E0700101)**

7. Khan, S.A., Nawaz, M.S., Khan, A.A. and Cerniglia, C.E. Direct in-gel hybridization of digoxigenin-labeled non-radioactive probes. Molecular and Cellular Probes, 13:233-237, 1999. Accepted: 3/10/99. **(E0690101)**

8. Khan, S.A., Nawaz, M.S., Khan, A.A. and Cerniglia, C.E. Simultaneous detection of erythromycin-resistant methylase genes ermA and ermC from *Staphylococcus* spp. by multiplex PCR. Molecular and Cellular Probes, Accepted: 7/22/99. **(E0700101)**

9. Khan, S.A., Nawaz, M.S., Khan, A.A., Steele, R.S. and Cerniglia, C.E. Characterization of erythromycin resistant *Staphylococcus* sp. isolated from mastitis infected milch animals. American J. Veterinary Research, Accepted: 8/12/99. **(E0690101)**

10. Lay, J.O., Holland, R.D., Rafii, F., Heinze, T.M., Sutherland, J.B. and Voorhees, K.J. Characterization of bacteria in spiked aqueous media based on MALDI TOF/MS of isolated biomarker proteins. Proceedings of the ASMS, Accepted: 6/14/99. **(Collaborating with Chemistry) (E0700501)**

11. Moody, J.D., Freeman, J.P. and Cerniglia, C.E. Biotransformation of doxepin by *Cunninghamella elegans*. Drug Metabolism & Disposition, 27:1157-1164, 1999. Accepted: 6/10/99. **(E0699901)**

12. Moody, J.D., Heinze, T.M., Hansen, E. and Cerniglia, C.E. Metabolism of the ethanolamine-type antihistamine diphenhydramine (Benadryl) by the fungus *Cunninghamella elegans*. Applied Microbiology and Biotechnology, Accepted: 9/2/99. **(E0694201)**

13. Nawaz, M.S., Khan, A.A., Khan, S.A., Paine, D.D., Pothuluri, J.V. and Cerniglia, C.E. Biochemical and molecular characterization of erythromycin-resistant avian *Staphylococcus* spp. isolated from chickens. Poultry Science, 78:1191-1197, 1999. Accepted: 3/31/99. **(E0690101)**

14. Parshikov, I., Freeman, J.P., Lay, J.O., Beger, R., Williams, A.J. and Sutherland, J.B. Regioselective transformation of ciprofloxacin to *N*-acetylciprofloxacin by the fungus *Mucor ramannianus*. FEMS Microbiology Letters, 177:131-135, 1999. Accepted: 6/15/99. **(E0705201)**

15. Parshikov, I., Freeman, J.P., Williams, A.J., Moody, J.D. and Sutherland, J.B. Biotransformation of *N*-acetylphenothiazine by fungi. Applied Microbiology & Biotechnology, 52:553-557, 1999. Accepted: 5/25/99. **(E0694201)**

16. Pothuluri, J.V., Freeman, J.P., Fu, P.P. and Cerniglia, C.E. Biotransformation of 1-nitrobenzo[e]pyrene by the fungus *Cunninghamella elegans*. Journal of Industrial Microbiology and Biotechnology, 22:52-57, 1999. Accepted: 12/15/98. **(E0699901)**

17. Rafii, F. Serratia. *In*: Encyclopedia of Food Microbiology. (Eds., Robinson, R.K., Batta, C.A. and Patel, P.) Academic Press, London, Accepted: 04/06/99. **(E0700701)**

18. Rafii, F., Lunsford, P., Hehman, G. and Cerniglia, C.E. Detection and purification of a catalase-peroxidase from *Mycobacterium* sp. Pyr-1. FEMS Microbiology Letters, 173:285-290, 1999. Accepted: 1/30/99. **(E0695901)**

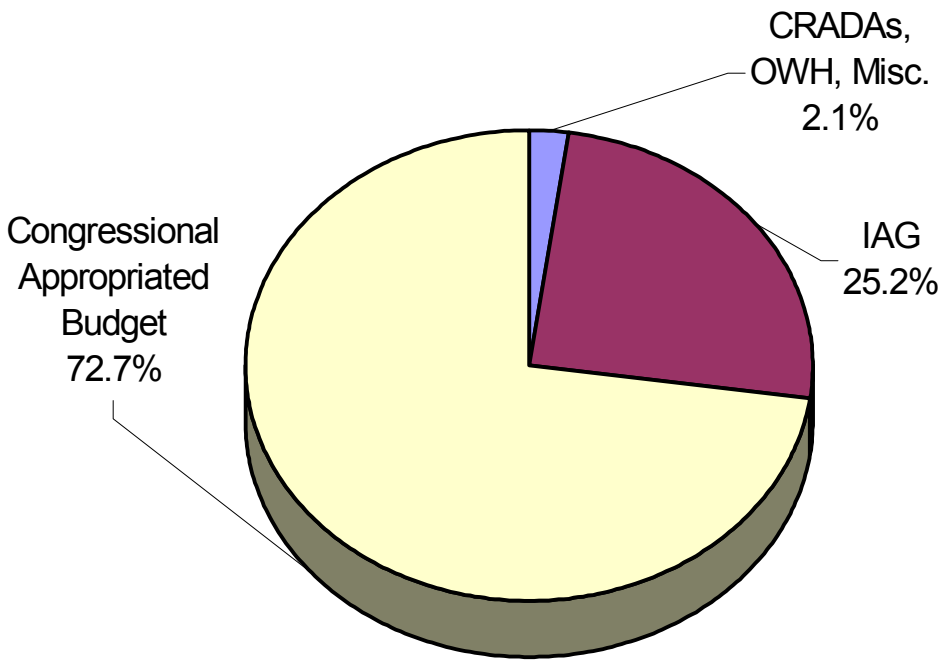
19. Rafii, F., Ruseler-Van Embden, J.H. and Van Lieshout, L. Changes in bacterial enzymes and PCR profiles of fecal bacteria from a patient with ulcerative colitis before and after antimicrobial treatment. Digestive Diseases and Sciences, 44:637-642, 1999. Accepted: 11/9/98. **(E0695901)**

20. Wang, R., Khan, A.A., Cao, W. and Cerniglia, C.E. Cloning, sequencing and expression of the gene encoding enolase from the fungus. *Cunninghamella elegans*. Appl. Environ. Microbiol., Accepted: 2/23/99. **(E0260201)**

21. Wang, R., Khan, A.A., Cao, W. and Cerniglia, C.E. Identification and sequencing of a cDNA encoding 6-phosphogluconate dehydrogenase from a fungus, *Cunninghamella elegans* and expression of the gene in *Escherichia coli*. FEMS Microbiol. Letters, 169:397-402,1998. Accepted: 10/27/98. **(E0260201)**

22. Wilkes, J.G., Letarte, S., Glover, K.L., Holcomb, M., Rafii, F. and Bertrand, M.J. In-Beam pyrolysis with a MAB-Tof instrument for rapid bacterial chemotaxonomy. Journal of Proceedings of ASMS 1999. Accepted: 6/14/99**. (Collaborating with Chemistry) (NA)**

SUMMARY OF EXTERNALLY FUNDED PROJECTS*



Relative Proportions of NCTR Budget

* Details of projects presented under individual Research Division sections.

INTERAGENCY AGREEMENTS (IAGS)

NCTR has been tenacious in establishing Interagency Agreements (IAGs) with other governmental agencies to conduct research on problems of common interest to FDA and the collaborating agency. The most significant, in terms of project magnitude and resource commitment, is the IAG between FDA/NCTR and the National Institute of Environmental Health Sciences (NIEHS)/National Toxicology Program (NTP). NCTR conducts animal bioassays, mechanistic studies, and risk assessments on a number of compounds of regulatory interest to both the FDA and the NIEHS/NTP. Within this IAG, NCTR has conducted studies on the following: 1) the mycotoxin, fumonisin B_1, a corn contaminant, which was nominated for study by the FDA Center for Food Safety and Applied Nutrition (CFSAN); 2) the pediatric sedative, chloral hydrate, nominated by FDA's Center for Drug Evaluation and Research (CDER); 3) malachite green, a therapeutic agent used in aquaculture, nominated by FDA's Center for Veterinary Medicine; and 4) the interaction of ethanol and urethane, nominated by CFSAN. Also, a mechanistic study on riddelliine, a compound of interest to CFSAN, is being supported by the FDA/NIEHS IAG.

NCTR has been tenacious in establishing Interagency Agreements (IAGs) with other governmental agencies to conduct research on problems of common interest to FDA and the collaborating agency.

Other research funded via the FDA/NIEHS/NTP IAG includes a series of studies on the endocrine-active compounds: genistein, ethinyl estradiol, nonylphenol, methoxychlor, and vinclozolin. The studies will determine the endocrine-disrupting effects of these compounds on reproduction, behavior, and carcinogenesis over multiple generations.

In addition to the studies on chloral hydrate mentioned above, the FDA/NIEHS/NTP IAG also funded an expansion of the chloral hydrate project to determine the effects of caloric restriction on the results of the standard bioassay. The results will provide information on whether controlled body weight gain will improve the longevity of the animals, decrease the incidence of tumors in control animals, and result in smaller standard deviations in the data observed in both control and test groups of animals, thus improving the precision of the bioassay.

As a result of CFSAN's concern about the potential interaction of UV light and over-the-counter cosmetics containing alpha-hydroxy acids, support for the development of a unique Phototoxicity Laboratory at the NCTR was received from NIEHS/NTP. Risk assessments of a number of FDA regulated products suspected of interacting with sunlight or fluorescent tube-generated lights began in FY1999 and are continuing.

The National Institute on Aging (NIA) has supported NCTR in conducting a broad area of research on the effects of caloric restriction on the extension of life span and the prevention of chronic diseases, including cancer. The project concluded in FY1999.

Although not an IAG in the strict sense, NCTR competed for and received funding from the FDA's Office of Women's Health (OWH) for a number of research projects addressing women's health concerns. These include: 1) the preparation of antibodies against UV photoproducts in support of the phototoxicity program; 2) the development of methodologies to assay hydroxylation of endogenous estrogens as that process relates to risk of developing breast cancer; and 3) research on the effects of nutritional folic acid deficiency in the etiology of spina bifida and Down syndrome.

The OWH also has provided generous support to NCTR for the development of a computerized predictive Endocrine Disrupter Knowledge Base (EDKB). This predictive system is nearing completion and soon will be available to other FDA organizations online via the FDA Intranet. The system, using both quantitative and qualitative delineators, will allow FDA to estimate the endocrine disrupting activity of a compound from its structure.

NCTR also has received support from both the FDA's Office of Women's Health and the U.S. Department of Defense (DOD) to conduct molecular epidemiology studies designed to determine the variability in metabolic phenotype and genotype in women with respect to their recurrence of breast cancer following high dose radiation and chemotherapy.

COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENTS (CRADAS)

NCTR scientists developed and licensed to Cox Recorders of Belmont, NC, a consumer-based, low-tech indicator of food freshness patented as Fresh Tag™. Cox Recorders is providing the means of producing one million tags per day and is assisting FDA in establishing the product's use in the food market more rapidly. This invention was selected as one of the "Best of What's New in 1999" by *Popular Science* magazine.

This invention (consumer-based, low-tech indicator of food freshness patented as Fresh Tag™) was selected as one of the "Best of What's New in 1999" by Popular Science.

NCTR's Division of Chemistry received financial support from Scientific Instruments Services, Ringola, NJ, to develop a universal interface for mass spectrometry analysis of high pressure liquid chromatography (HPLC) effluent containing chemicals and metabolites.

NCTR has received support via a CRADA with Genometrix to develop a "Risk-Tox DNA micro-array chip" for rapid , high throughput genotyping. The results of this CRADA will provide FDA the ability to genotype patients for all the major enzyme variants that would predict susceptibility to carcinogens, adverse drug reactions, chemotherapeutic drug efficacy, and allow individualized dosing of therapeutics.

Both the Chemical Manufacturers Association (CMA) and FDA's Office of Women's Health have provided NCTR support for the development of a computerized predictive Endocrine Disrupter Knowledge Base (EDKB). Using both quantitative, i.e., Structure-Activity Relationships (QSAR), and qualitative methodology, the EDKB will be able to screen chemical structures for endocrine disrupting activity and will serve as a prototype for predicting activity of other chemical classes such as androgens and thyroid hormones and may be applied to other toxic endpoints such as neurotoxicity and carcinogenesis.

NCTR's Division of Neurotoxicology has received financial support from AstraZeneca to study the effects of long-term blockage of glutamate receptors and/or sodium channel blockage on neurobehavioral endpoints in the non-human primate.

UNIVERSITY INTERACTIONS

NCTR senior scientists hold adjunct faculty positions and collaborate with individuals and departments of several universities. This practice has been instrumental in leveraging both the intellectual and infrastructure capabilities of NCTR. NCTR scientists have developed research collaborations with more than 20 universities and many NCTR scientists have been granted adjunct academic positions. This arrangement permits NCTR staff to develop close collaborative efforts with various university staffs to solve problems of mutual interest to FDA and the respective university. Academic collaborations include mutual use of specialized equipment, sharing of research samples to maximize the gain of information from a project, and the exchange of staff between the two institutions for lectures, seminars, and conduct of research.

Collaborations between NCTR scientists and universities in the United States and abroad have resulted in, at no cost to FDA, a vigorous program of visiting scientists who come to NCTR to pursue research in areas developed by NCTR.

Of particular importance are the close collaborations between NCTR and the University of Arkansas for Medical Science (UAMS) in Little Rock, AR. In addition to the adjunct positions held by NCTR scientists at UAMS, NCTR participates in the UAMS Interdisciplinary Toxicology Program through which graduate students receive a Ph.D. in toxicology. Many of the graduate students perform research for their dissertations in an NCTR laboratory under NCTR staff supervision.

Another example of leveraging with local institutions is that NCTR staff in the Division of Neurotoxicology developed a behavioral test battery that is able to detect neurological deficits in non-human primates and rodents and in collaboration with physicians and patients at the Arkansas Children's Hospital (ACH) have shown that this test battery can detect neurological deficits in children. This strengthens the risk assessment of and risk decisions resulting from the use of animal tests on compounds regulated by FDA.

Collaborations between NCTR scientists and universities in the United States and abroad have resulted in, at no cost to FDA, a vigorous program of visiting scientists who come to NCTR to pursue research in areas developed by NCTR scientists. During FY1999-2000, NCTR will host more than 45 visiting scientists from the U.S. and 14 foreign countries. These visiting scientists not only contribute valuable scientific expertise to NCTR research programs, but many return to their respective institutions to continue research on problems of interest to NCTR and FDA and their home country.

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